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SEASONAL DEVELOPMENT IN THE NURSERY OF DAMPING-OFF OF RED PINE SEEDLINGS CAUSED BY PYTHIUM AND RHIZOCTONIA[1]

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INTRODUCTION

A detailed study of the weather and certain soil conditions in relation to damping-off of red pine seedlings in Wisconsin forest nurseries has been undertaken. The present paper is the third of a series on the damping-off of red pine seedlings by *Pythium* and *Rhizoctonia*. In the first paper (*6*) [3] the causal agents were shown to be *Pythium irregulare* Buisman and *Rhizoctonia solani* Kühn and their distribution, symptoms caused, and life histories were presented; in the second paper (*7*) the influence of controlled temperature, moisture, and soil reaction was described. Several interacting factors have operated at various times and in different places to favor or to inhibit damping-off in the nursery. The study here reported attempts to clarify the outcome when all these factors act freely together in nature.

The reaction of the soil has appeared to be a very important influencing factor. Hartley (*3*) has observed that in the nursery severe damping-off was highly correlated with neutral or only slightly acid soils. Damping-off was not serious on acid soils where the pH was less than 6. Roth and Riker (*7*) have reported that damping-off is somewhat diminished in the greenhouse in soils of about pH 5 but that deviation from this reaction in either direction may increase the severity of attack where both *Pythium* and *Rhizoctonia* are the causal fungi.

High soil moisture and air humidity, it is commonly assumed, favor damping-off. This view was partly upheld by Hansen et al. (*2*), who studied damping-off in seedbeds receiving different amounts of water. They found that, in general, but not always, heavy watering increased the amount of damping-off. C. Roth (*5*) considered wet soil and high air humidity favorable to damping-off by *Rhizoctonia* during periods of hot weather. However, Hartley (*3*) observed that in a Nebraska nursery damping-off decreased after heavy rains and did not increase again until soil moisture had gone down. Roth and Riker (*7*) found that various damping-off fungi responded differently in the greenhouse to soil moisture, *Pythium* thriving in wet soil and *Rhizoctonia* causing greater losses in somewhat drier soils and at saturated air humidity.

That high temperatures favor damping-off was pointed out by Jones (*4*), Hartley (*3*), and C. Roth (*5*). Hartley and C. Roth interpreted their data by placing the values for total damping-off at differ-

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[3] Italic numbers in parentheses refer to Literature Cited, p. 431.

ent intervals after emergence in juxtaposition to meteorological records obtained during the same period. Hartley counted damped-off seedlings twice daily in naturally infested beds, but in presenting his data he made no statement regarding the kinds of damping-off fungi present. C. Roth's (*5*) studies were made on beds inoculated with *Pythium*, *Fusarium*, and *Corticium* (*Rhizoctonia*), but his discussion of results mentioned only *Corticium* damping-off. In greenhouse studies in soils inoculated with *Pythium* and *Rhizoctonia*, Roth and Riker (*7*) found that warm temperatures favored damping-off by both fungi. When the soil was wet, *Rhizoctonia* was strongly inhibited by cool temperatures while *Pythium* continued to cause severe losses.

Clarifying the effect of environmental factors on disease development involves several considerations: (1) Temperature and moisture often vary simultaneously, to complicate results. Field plots can seldom be controlled to the point where an effect may be attributed to a single variable. (2) The reaction of the soil influences the activity of the causal fungi, *Rhizoctonia* predominating in soil more acid than approximately pH 5.8 and *Pythium* in that less acid. (3) The identity of the active dumping-off fungi must be known since the various causal agents differ (*5*, *6*) in their response to environment. Information that has been obtained where one organism is acting cannot be applied where the loss is caused by a different organism. (4) Age of the host plant strongly affects its susceptibility (*3*), prohibiting direct comparison of the results of one count with those of a subsequent count of the same stand. (5) Age differences influence the time required for symptom development (*3*). Thus, in older seedlings when counts are made at frequent intervals, the effect of an attack one day may not appear until a day or more later. (6) Various host plants differ in susceptibility (*3*, *9*) while various strains of the fungi differ in pathogenicity (*3*, *6*). Results obtained with a particular host and parasite must, therefore, be applied with reservation to a different disease situation.

With these various considerations in mind the writers have examined the seasonal expression of damping-off in a nursery in central Wisconsin and have interpreted the relative importance of the different interactive influences involved. It appeared that a clarification of interacting environmental factors might help explain the variation in the occurrence of damping-off and in results secured with different control measures from year to year in the same nursery and from nursery to nursery during the same year.

1938 EXPERIMENTS

MATERIALS AND METHODS

The experimental area was a uniform nursery block, provided with overhead irrigation and located some distance from roads or windbreaks. It had been reclaimed from an abandoned field in 1932 and seeded to white pine in 1933. Damping-off in this first planting was so severe that the area was subsequently used only for transplanting. The soil was Plainfield sand of uniform structure and low but uniform fertility. The reaction was pH 5.5.

Sixteen standard 4- by 12-foot seedbeds were placed in a rectangle with 4 beds to a side. Each bed accommodated five 2- by 4-foot ex-

perimental plots having 6-inch separations between plots. This arrangement allowed 4 replications of 20 treatments (seeding dates). The replications, each consisting of 1 row of 4 beds, were placed side by side, and the 20 plots within each replication were completely randomized. Each bed was covered with a bird- and rodent-proof frame providing half shade.

The soil of each 2- by 4-foot plot was uniformly inoculated by thoroughly mixing into the upper 3 inches one-third quart of a corn meal and sand culture of *Rhizoctonia* (F–5) and a similar amount of *Pythium* (F–111–A), described earlier (*6*). The plots were seeded 5 days after inoculation.

Seed employed in 1938 and 1939 was from a uniform, locally produced lot of red pine (WR–17). It germinated 91.3 percent in a bed of sterile sand adjacent to the experimental plots.

Complications arising from age differences of the host were reduced by providing throughout the summer a population of seedlings of uniform age. This supply was obtained by seeding a new set of four plots (one in each replication) every 5 days.

Approximately 1,450 seeds were planted in each plot consisting of twelve 4-foot drills. The 2 outer drills and 3 inches of the ends of the 10 inner drills were omitted from counts to avoid marginal effect. Counts were taken on 20 feet chosen by marking 2 linear feet from alternate ends of each of the 10 inner drills of the plots and included about 550 viable seeds.

Meteorological records were made at the plots throughout the summer. Instruments used were a standard drum-recording hygrothermograph and a drum-recording soil thermograph with the bulb buried one-quarter inch in one of the experimental plots. Rainfall and water thrown by the overhead sprinklers were measured with a standard rain gage. The moisture content of the uppermost inch of soil was measured daily at 8 a. m. in three composite samples taken at random from the experimental area. The soil reaction was measured periodically on composite samples with the quinhydrone electrode. Occasional samples were also tested with a glass electrode to check the efficiency of the other instrument.

The experiment was so designed that seedlings in the four plots of the first seeding were counted 5 days after emergence and, thereafter, at 5-day intervals until six counts had been made. The seedlings had then passed their age of practical susceptibility. The second and later seedlings were treated in like manner throughout the summer. Thus a succession of damping-off and survival counts was provided of seedlings comparable in age but variously affected by environment. Preemergence damping-off has been omitted from the records of results. A series of estimates indicated that in the field preemergence damping-off had approximately the same ratio to postemergence damping-off as under similar but controlled conditions in the greenhouse (*7*).

A representative sample of damped-off seedlings was collected on each counting date from the plots of each age group. A section of the hypocotyl from each seedling was plated and the causal fungus determined. The numbers of seedlings plating *Pythium* and *Rhizoctonia*, respectively, were recorded. The causal fungi could be consistently isolated only from seedlings damped off 1 to 3 days preceding

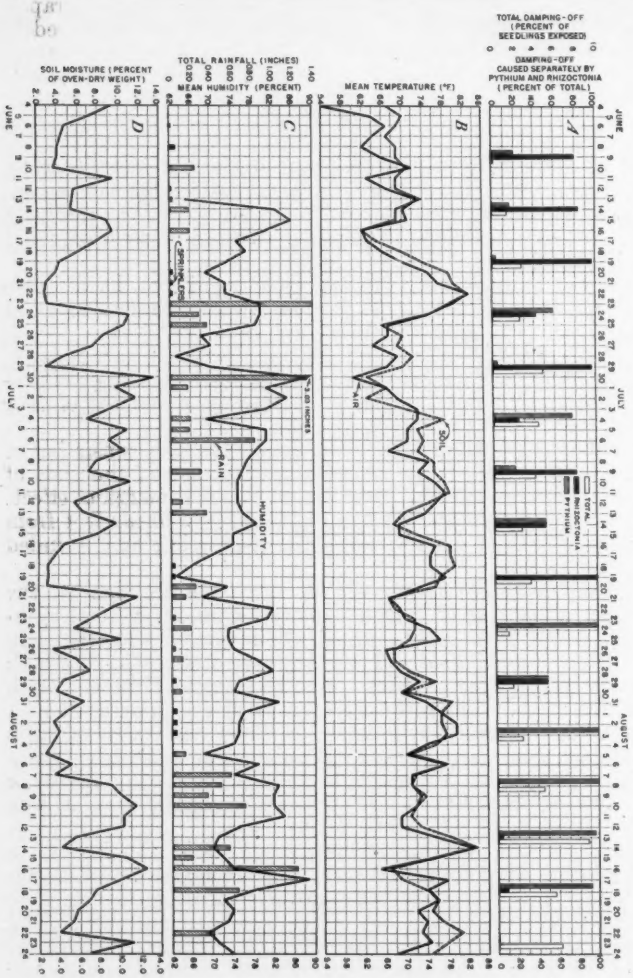

FIGURE 1.—Damping-off of red pine caused, respectively, by *Pythium* and *Rhizoctonia* during the summer of 1938 in inoculated field plots on Plainfield sand with a pH of 5.5: *A*, Damping-off for consecutive 5-day periods in percent of seedlings exposed, and percent of this total loss caused, respectively, by *Pythium* and *Rhizoctonia; B*, mean daily air and soil temperatures; *C*, total daily precipitation, including water thrown by Skinner irrigation, and mean daily humidity in percent; *D*, daily soil moisture at 8 a. m. expressed as percent of the oven-dry weight of the soil.

the count. Therefore, while the "total" columns in the graphic presentation of the data (fig. 1) represent all seedlings damped-off during the 5-day period, those in the *Pythium* and *Rhizoctonia* columns were secured chiefly from seedlings attacked during the 3 days immediately preceding the count.

EXPERIMENTAL RESULTS

The records of the 1938 studies have been summarized and are presented in graphic form in figure 1. The amount of damping-off is given in the uppermost portion (*A*) while records of temperature (*B*) and moisture (*C* and *D*) appear below in juxtaposition for easy comparison. The amount of damping-off during 5-day intervals is given as the percent of the total number of seedlings in all age classes. Thus on July 9 it appears that, since July 4, 4 percent of all the seedlings in six different age classes (0 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, and 25 to 30 days after emergence) damped-off. Of this 4-percent loss, 80 percent was caused by *Rhizoctonia* and 20 percent by *Pythium*.

Slight variation in age classes appeared in the first two records. On June 9 the only seedlings recorded were those from classes 0 to 5, 5 to 10, and 10 to 15 days after emergence. On June 14, only seedlings from the five age classes 0 to 5, * * *, 20 to 25 days were included. On June 19 and all subsequent dates seedlings from the six age classes 0 to 5, * * * , and 25 to 30 days were included. There was no record for August 23 of the kind of fungi responsible for damping-off.

In general, damping-off increased throughout the summer with rising temperature. A broad survey of the season shows that the sum of damping-off losses recorded for all counting dates in June, each representing total damping-off in all age classes, was approximately 11 percent; in July, 17 percent; and in August, 29 percent. The mean temperatures for the 3 months, respectively, were 67°, 71°, and 73° F. However, there were occasional conflicting fluctuations within the various 5-day periods, such as the decline in amount of disease near the end of July. This decline accompanied and was probably conditioned by cooler weather and dryer soil.

On relative losses caused by *Pythium* and *Rhizoctonia* the influence of temperature and soil moisture was conspicuous. For the season as a whole 54 percent of the total loss was caused by *Pythium* and 46 percent by *Rhizoctonia*. The two organisms were not, however, equally destructive at all times throughout the season, *Rhizoctonia* being the more important after emergence during the spring and *Pythium* during the summer. For the months of June, July, and August *Rhizoctonia* caused 79, 50, and 4 percent, respectively, of the total loss; *Pythium* 21, 50, and 96 percent. Since temperatures increased from month to month, the figures suggest correlations between warm temperature and *Pythium* damping-off after emergence, cooler temperature and *Rhizoctonia* damping-off. The almost complete disappearance of *Rhizoctonia* during and after the hot, dry August weather is conspicuous. Since it operates almost entirely near the surface of the soil, the question arises whether the sun may have had a partially sterilizing effect on the soil surface. *Pythium* commonly operates at lower levels.

A set of means computed for the temperature throughout the season showed that here somewhat raised temperatures favor *Pythium* over *Rhizoctonia*. (See fig. 1, June 24, July 4, July 24, August 3, and August 18.) The range of temperatures, however, was not great—from 74° to 69° F.

Several inconsistencies, nevertheless, did occur. There were severe *Pythium* losses during periods of moderately cool temperature on August 8 and 13, and attacks by *Rhizoctonia* diverged even more from the general correlations of temperature and loss. Severe *Rhizoctonia* losses on July 9 and 19 followed periods of warm temperature while those on June 9, 19, and 29 occurred during cool periods.

Although temperature appeared generally to affect the ratio of *Pythium* to *Rhizoctonia* damping-off, the results for soil moisture were more evident and more consistent. The influence of soil moisture on the relative activity of *Pythium* and *Rhizoctonia* was more clearly shown by examination of figures for the individual 5-day periods than by comparison of the monthly records. As explained earlier, isolations were made from seedlings damped-off only 1 to 3 days before collection. Thus, the material used for isolations July 19 came from plants damped-off during the dry period of July 17, 18, and 19. In this and most other cases (August 3 being a striking exception) *Rhizoctonia* predominated after relatively dry periods and *Pythium* after relatively moist periods. The severe attack by *Pythium* in dry soil on August 3 probably occurred because of the warm temperature during the preceding 3 days. For the seven 5-day periods (isolation periods) during which *Phythium* loss exceeded *Rizoctonia* (fig. 1), the mean soil moisture was 7.2 percent; when *Rhizoctonia* predominated, it was only 5.4 percent.

The 1938 results suggested that while temperature may affect the relative importance of the two fungi, it has a greater influence on total damping-off. Soil moisture, on the other hand, affected more strongly the relative losses caused by the two fungi than it did the total loss. The air-humidity curve in this experiment so closely paralleled that for soil moisture that its influence upon damping-off could not be distinguished.

1939 EXPERIMENTS

The 1938 studies were followed during the summer of 1939 by experiments designed to check more closely on various important points. Improvements made in the 1939 investigations over those in 1938 consisted chiefly (1) of shortening the interval between consecutive counts; (2) of increasing the number of plots so that larger samples of damped-off seedlings would be available for plating and determining the ratio of *Pythium* to *Rhizoctonia*; (3) of maintaining a high soil-moisture content in one of two series of plots to clarify temperature and moisture effects; and (4) of providing plots with different soil reactions.

MATERIALS AND METHODS

The 1939 experiment was conducted on the site used in 1938. Forty 4- by 12-foot seedbeds were placed in 10 rows of 4 beds each between the overhead pipe lines. There were 5 replications and 20 experimental plots, measuring 4 by 4½ feet, that were randomized

within each replication. As in the 1938 experiments, these plots represented different seeding dates. However, in 1939 a split-plot design was used in which one-half of each plot was hand-watered daily and the other half given only normal water from rain or the overhead sprinklers. The "normal" half sections were thus identical with the full plots of the 1938 experiment. Inoculation, seeding, and counting of postemergence damping-off were done as in 1938. Similarity of treatment made possible direct comparisons between normal plots during the two seasons as well as between watered and normal plots in the 1939 season.

The watered half-plots were sprinkled daily between 2 and 4 p. m., except on a day of heavy rain or the day following. Water to the equivalent of about 0.2 inch was uniformly applied to each plot.

In taking disease data, figures representing total damping-off and survival, with the plating results from seedlings collected at the different counts, were tabulated as in 1938. Inoculation, seeding, and counting were repeated at 3-day rather than 5-day intervals. Consequently, before the seedlings in any set of plots had stood the 30 days required for relative immunity, they had been counted 10 rather than 6 times.

The weather records in 1939 were kept differently from those shown in figure 1, where the 5-day sums for damping-off were placed parallel to the mean daily weather records. In the 1939 experiments the mean 3-day weather records were calculated and placed opposite the 3-day values for damping-off. Each point on the weather curves in figure 2, therefore, represents the mean of the 3-day period indicated. The bar graphs for rainfall, however, show the totals for the 3-day periods.

The reaction of the soil of the experimental area was changed from about pH 5.5 to 7.0 by thorough mixture of lime with the soil at the rate of 1 ton per acre the fall before seeding. As the season progressed, the acidity drifted to about pH 6.3. This treatment was performed to increase the severity of disease in accord with the opinion of various writers (1, 3, 5, 8) that damping-off is favored by neutral or only slightly acid soils. The results appear in figure 2. During the latter part of July, 1939, an additional companion experiment was set up on soil adjacent to the limed plot. The soil had a reaction of pH 5.5, similar to that employed in 1938. The size and treatments were identical with those of the normal halves of the limed plots. Comparisons showed which of the differences observed between the 1938 and 1939 results were attributable to change in soil reaction and which to seasonal differences. The results are given in figure 3, where the dates and damping-off results at pH 6.3 are the same as those on the right side of figure 2. The corresponding weather data are not repeated, and only the curve for *Rhizoctonia* damping-off is shown. The *Pythium* loss would be represented by a "mirror image" of this curve like that in figure 2, *A*.

EXPERIMENTAL RESULTS

Seasonal Trends

Certain broad seasonal trends will be considered before examining detailed results as influenced by fluctuations in weather. Total damping-off in the normal plots did not differ significantly for the two

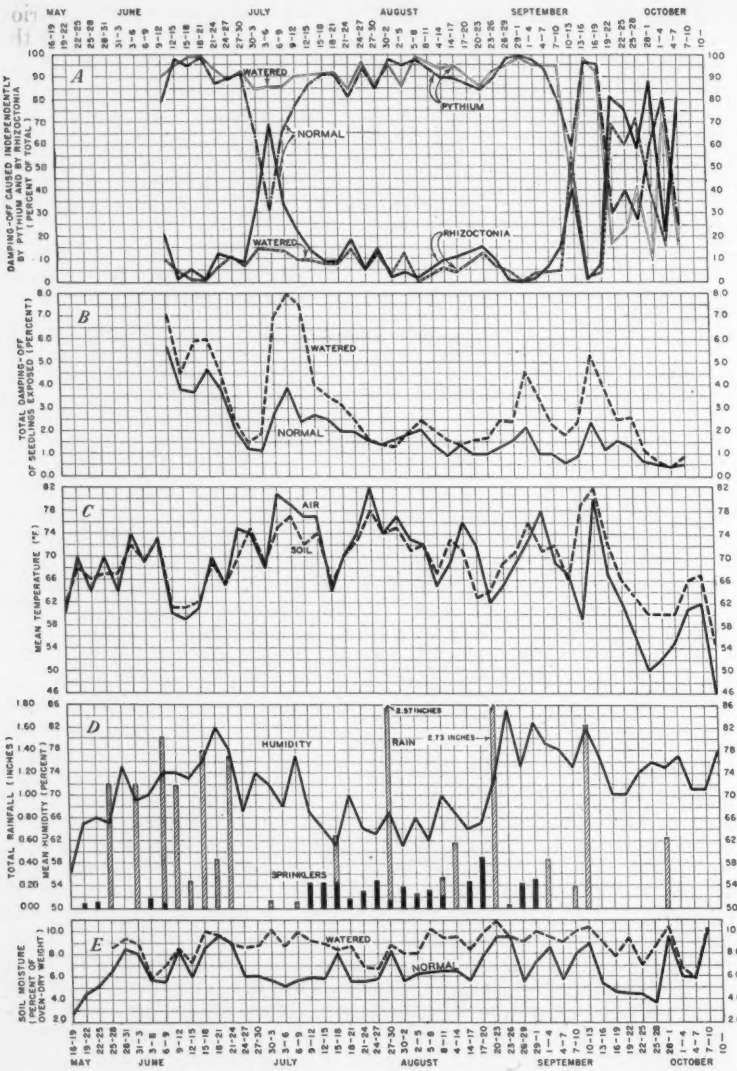

FIGURE 2.—Damping-off of red pine caused, respectively, by *Pythium* and *Rhizoctonia* during the summer of 1939 in inoculated, watered, and "normal" (no special watering) field plots with a pH of 7.0 to 6.3: *A*. Damping-off in watered and normal plots caused independently by *Pythium* and *Rhizoctonia*, expressed as percentage of total loss (thus the loss caused by *Pythium* is the "mirror image" of that by *Rhizoctonia*); *B*, total damping-off for consecutive 3-day periods in percent of seedlings exposed; *C*, mean 3-day air and soil tempera tures (° F.); *D*, sum of 3-day precipitation, including water thrown by Skinner irrigation, and mean 3-day humidity in percent; *E*, mean 3-day soil moisture in watered and normal plots expressed as percentage of the oven-dry weight of the soil. Results in more acid (pH5.5) soil appear in figure 3.

seasons. The mean monthly damping-off in 1938 for the period including June, July, and August was 18.9 percent while that for the corresponding period in 1939 was 19.1 percent. The loss in the watered plots, however, in 1939 (30.6 percent) was much greater than in the normal plots during either season. In 1939 the loss was greatest in the spring, becoming progressively less severe as the summer advanced. In 1938, except for the period of light attack late in July, damping-off increased as the summer progressed.

The change in soil reaction appeared not to influence the total loss for the two seasons. Liming, strongly favorable to *Pythium* and

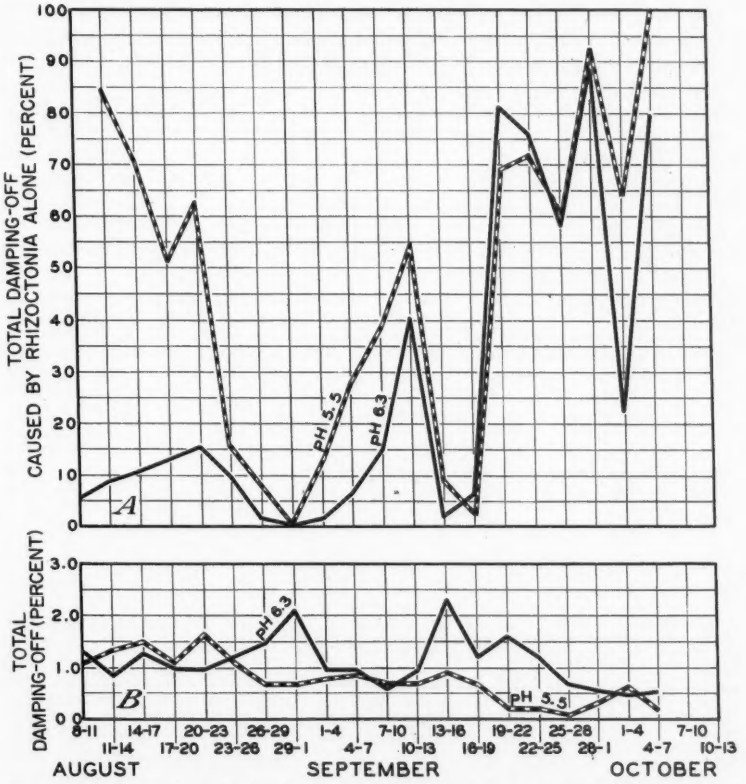

FIGURE 3.—Damping-off of red pine caused by *Pythium* and *Rhizoctonia* between August 8 and October 7, 1939, in inoculated, "natural" field plots in two experiments with respective soil reactions of pH 5.5 and 6.3: *A*, Damping-off caused independently by *Rhizoctonia* expressed as percent of the total loss; *B*, total damping-off for consecutive 3-day periods in percent of seedlings exposed. Other factors influencing the loss were the same as in figure 2 between August 8 and October 7. To avoid confusion of additional lines in *A*, the curves for *Pythium* have been omitted. Since curves for both fungi are based on those in *B*, the *Pythium* curves not shown would be complements of the *Rhizoctonia* curves in *A*. The meteorological conditions are the same as those in figure 2 at corresponding dates.

inhibitory to *Rhizoctonia*, resulted in a reduction of the *Rhizoctonia* loss with a corresponding increase in the number of seedlings killed by *Pythium*. In table 1 are given the relative amounts of damping-off caused independently by the two fungi at different soil reactions and moistures. In normal plots of 1938 with a pH of 5.5, the ratio of losses caused by *Pythium* and *Rhizoctonia*, respectively, was about 1:1. In 1939, after adjustment of the reaction of this same area to pH 7.0, the ratio shifted to about 4:1 in the normal plots and to slightly over 5:1 in the watered plots. During the 2-month period from August 8 to October 7 (the period during which the third experiment was run) the damping-off ratio in the normal plots with a pH of 7.0 was approximately 2.5:1. This value was reduced somewhat from that for the season as a whole because of cool autumn temperatures and low soil moistures which inhibited *Rhizoctonia* less than *Pythium*. However, when the soil reaction was at pH 5.5, return of the ratio to about 1:1 indicated that the differences in the ratios were caused by changes in soil acidity. That temperature and humidity over the ranges encountered were not important in influencing the relative losses caused by *Pythium* and *Rhizoctonia* in the different experiments was suggested by the slight differences in these factors during the two seasons. In 1938 the mean air temperature for the experimental period was 67° F. while that for 1939 was 66°. Humidity during the two seasons was 75 and 74 percent, respectively.

TABLE 1.—*The influence of soil reaction upon the percent of total damping-off caused independently by Pythium and Rhizoctonia in inoculated field plots*

Season and date	Treatment	Soil reaction	Soil moisture	Inoculation made with—	Total damping-off caused by each fungus
1938		*pH*	*Percent*		*Percent*
June 4 to August 24	Normal	5.5	7.1	*Pythium*	53
				Rhizoctonia	47
1939					
June 9 to October 7	Normal	7.0	6.5	*Pythium*	79
				Rhizoctonia	21
	Watered	7.0	8.7	*Pythium*	83
				Rhizoctonia	16
1939					
August 8 to October 7	Normal	7.0	6.6	*Pythium*	72
				Rhizoctonia	28
		5.5	6.6	*Pythium*	51
				Rhizoctonia	49

The effects of temperature noted in 1938 appeared also in 1939. Mean damping-off for the months of June, July, August, and September, respectively, was 3.5, 2.2, 1.5, and 1.2 percent while the corresponding mean air temperatures were 68°, 75°, 70°, and 65° F. Excluding the month of June, there was a progressive decrease in damping-off through the summer with declining temperature. The high damping-off values during June can in large part be attributed to high susceptibility of the seedlings counted during that period rather than to weather conditions alone. All seedlings counted before June 24 (the date at which seedlings of all age classes were included) were approximately 9 days younger in mean age than seedlings counted at all subsequent dates. Their susceptibility was correspondingly greater, as discussed earlier.

The amount of soil moisture in 1939 showed no significant correlation, as in 1938, with the monthly trend of damping-off. However, comparison of the watered and normal plots in 1939 (fig. 2, *B* and *E*) showed that soil moisture greatly influenced total damping-off. In the watered plots, where no dry periods occurred, there was a seasonal mean of 31 percent. In the normal plots a comparable mean was only 19 percent. On only one counting date, August 2, did damping-off in the dry sections exceed the loss in the watered sections of the plots.

These relatively broad observations, dealing with conditions during seasonal or monthly periods, are valuable for indicating trends. However, the actual situations are clarified by studying, through many successive short-time intervals, the changing factors of environment and the corresponding variations in the development of disease.

Short-Time Fluctuations in Weather

The importance of temperature in the damping-off relationship appears conspicuously in figure 2. It is seen (*B*) in watered plots, provided humidity and soil moisture are high (*E*), that greatest damping-off commonly corresponds with warmest temperatures (*C*). These are conditions of temperature and moisture favoring *Pythium* damping-off, and in view of the neutral soil reaction, a severe loss would be expected. The peaks, e. g., on June 18 to 21, July 6 to 9, and so on, for total damping-off in the normal plots are not so high but correspond with those in the watered plots.

High air humidity and surface moisture (*D*), concurrent with high temperature, correspond with the 3 pronounced peaks of damping-off (*B*) on July 6 to 9, August 29 to September 1, and September 13 to 16. With low humidity the important top one-quarter inch of soil dried rapidly. The humidity was low during those periods of high temperature, July 24 to 27 and August 14 to 17, when damping-off was at a minimum. The relation of high air humidity to damping-off by *Rhizoctonia* has been discussed earlier (*7*).

The influence of soil reaction is seen from a comparison of the results given in figure 3. Total damping-off in the acid soil (pH 5.5) is seen in the latter figure to be sometimes greater and sometimes less than that in the relatively neutral soil (pH 6.3).

Apparently the relative activity of *Pythium* and *Rhizoctonia*, as well as the severity of total damping-off, was influenced especially by soil reaction, temperature, and moisture. In some cases these factors evidently supplemented each other in favoring one of the fungi and discouraging the other.

Changing Weather and Fungus Activity

In soils with different acidities (fig. 3) the variations in the amount of damping-off are clarified only when the causal agents and the changing weather (see fig. 2 with corresponding dates) are taken into account. As shown in figure 3, *Rhizoctonia* was more active than *Pythium* in acid soil (pH 5.5) from August 11 to 20, September 10 to 13, and September 19 to October 4. *Pythium* predominated in soil with pH 6.3 as late as September 19 and appeared prominently again October 1 to 4. From August 20 to September 10 and September 13

to 19 temperature and moisture appeared so favorable for *Pythium* that they overbalanced the relatively unfavorable acidity of the soil with pH 5.5.

Temperature and moisture effects are so intimately associated that they are considered together. Their fluctuations influenced the ratio of *Pythium* to *Rhizoctonia* damping-off in both the watered and the normal plots. In the watered plots about 90 percent of the total postemergence loss (fig. 2) was caused by *Pythium* until cool fall weather arrived. From September 10 to 13 the two fungi were about equally active; after September 19 *Rhizoctonia* remained generally

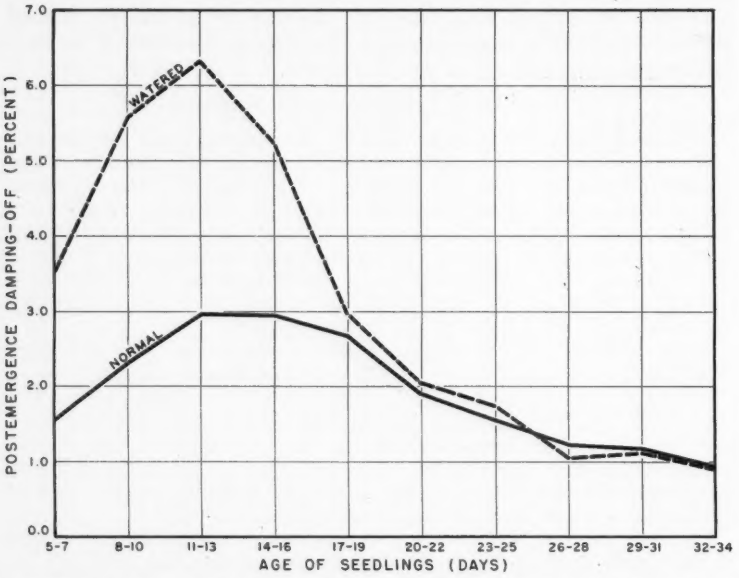

FIGURE 4.—Influence of seedling age on susceptibility of red pine to damping-off by *Pythium* and *Rhizoctonia* inoculated into watered and normal field plots.

predominant against emerged seedlings with the continued low temperatures.

In the normal plots the fluctuations in activity by the two fungi were greater than in the watered plots. In general, high soil moisture, high humidity, and warm temperature, when acting together, strongly favored attack by *Pythium* (e. g., fig. 2, June 18 to 21, August 19 to September 4, and September 13 to 16). The inhibition of *Rhizoctonia* activity by abundant soil moisture was indicated by the difference in amount of disease between watered and normal plots and the marked drop after the rain of October 1. However, high air humidity may be associated with severe *Rhizoctonia* attack (fig. 2, July 3 to 9). By encouraging aerial mycelium to grow over the seedlings, it may prompt the most destructive action of this fungus.

In general, it appeared that *Pythium* operated best with relatively wet, not too acid soil and warm temperature while *Rhizoctonia* was

more active in relatively acid soil with low moisture and a cool temperature. A single factor or a combination of two or more factors operated at times to inhibit one fungus and to encourage the other.

Seedling Age and Susceptibility

The varying relation of seedling age to susceptibility is indicated in figure 4. Records were taken at 3-day intervals on the untreated and watered seedlings studied in figure 2. As explained earlier, these had been planted at 5-day intervals throughout the season so that the question of seedling age would not influence the other results. In both watered and normal plots more seedlings damped-off 11 to 13 days after emergence than at any other age. After 13 days in the watered plots there was a sharp decline with increasing age until 17 to 19 days. At greater ages the loss became gradually less pronounced until at 32 to 34 days it fell below 1.0 percent. In the normal plots, after 11 to 13 days, damping-off decreased gradually toward 32 to 34 days. The losses in both normal and watered plots were similar after 3 weeks.

If the seedlings escaped disease for 30 days after emergence, the typical symptoms of damping-off became less and less evident. Although the pathogenic fungi continued to operate under very favorable conditions, the symptoms were commonly those of root rot and seedling blight and hence beyond the scope of this study.

DISCUSSION

The variations in results reported by various authors and mentioned in the introduction have suggested that several factors of unknown influence were involved in the circumstances resulting in the damping-off of red pine seedlings. As explained earlier (*6*), two organisms seemed to be the primary causal agents in Wisconsin. In another study (*7*) temperature, moisture, and soil reaction were observed to influence the activity of both these causal agents. For the most part, within limits, the environment that discouraged one favored the other. This indicates the difficulty of disease prevention by regulating the more obvious environmental factors.

The results of greenhouse studies under controlled conditions correlated closely with results secured in the field under natural conditions. Therefore, the influence of temperature, moisture, and soil acidity, operating in various combinations in the field, could be more exactly interpreted on the basis of the way that it was found to operate in the greenhouse. In making such interpretations, several conditions of study must be explained: (1) In the field studies only postemergence damping-off was considered while taking the records. An estimate of the preemergence damping-off in the field was made and found to correlate closely with that secured in the greenhouse. (2) The time at which the different records were made and averaged for the points indicated in the several figures sometimes caused an appearance of discrepancies. For example, during the period July 27 to 30 there was over 2½ inches of rain. During this period the relative humidity averaged about 66 percent. Obviously, during the heavy rainy period the humidity approximated 100 percent. (3) When meteorological conditions had been unfavorable for the growth

of fungi in the soil, fungus attack on the seedlings, as might be expected, lagged for some time after conditions again became favorable. (4) A consideration of weather conditions most favorable for active seedling growth involved such factors as have been explained earlier—a soil reaction between pH 4.5 and 6, soil moisture neither too dry nor too wet, and temperature between 18° and 30° C.

Variations in weather during the critical 30 days following plantings are of primary interest to the nurseryman. The attempt to secure as wide as possible a variation in weather but comparable conditions otherwise presented serious problems. Since variations occur very rapidly within the same season, it was considered that successive plantings throughout two seasons might have a reasonable chance of encountering practically all of the conditions a nurseryman might encounter in the springtime. If one examines the weather records, he finds all variations of temperature and moisture in different combinations, acting at a time when the seedlings are fully susceptible. From the results under a given set of conditions, he may secure a reasonable approximation of what may happen if that kind of weather occurs as his seedlings are emerging.

Most of the discrepancies reported in the introduction have been clarified by showing the presence of two diseases, caused by separate agents, and appearing, respectively, under different environmental conditions. The value of fall planting is consistent with the fact that the seedlings ordinarily emerge very early in the springtime, when temperature suppresses the activity of *Pythium* and high soil moisture represses *Rhizoctonia*. The value of the sulfuric acid soil treatments is apparently increased beyond partial surface disinfection because soil acidity is unfavorable to *Pythium* and soil moisture is often too high for vigorous *Rhizoctonia* development, particularly where the beds are not covered with sterile sand.

Detailed information about the circumstances under which these two fungi operate indicates some requirements for developing control measures.

SUMMARY

The influences of temperature, soil moisture, air humidity, and soil reaction upon damping-off of red pine have been studied in Wisconsin nurseries. The experimental plots were inoculated with local strains of *Pythium* and *Rhizoctonia*, the causal agents of the disease in Wisconsin. The relative importance of the various factors was continually changing with natural variations in the weather. Data in the different experiments were taken at 5- and 3-day intervals throughout two seasons on equal numbers of seedlings. Seed was planted and counts were made at regular time intervals in order that the effects of environmental conditions might be observed on seedlings of comparable ages. Only postemergence damping-off was considered in the records.

The highest percentage of damping-off occurred in seedlings emerged about 11 to 13 days. They were relatively resistant after about 1 month.

The weather often determined which of the two fungi was more active in soil with a reaction between pH 5.5 and 7.0. One or the

other might be inhibited, but no natural condition favoring seedling growth inactivated both at the same time.

Variations in soil reaction between pH 5.5 and 7.0 had little influence on total damping-off. A soil reaction of pH 5.5 to 6.0 was favorable to both fungi. More acid soils encouraged *Rhizoctonia* while *Pythium* was favored by soil more nearly neutral.

In general, temperature was more important in determining severity of total damping-off while soil moisture determined which of the two fungi would predominate. Except for early preemergence and some postemergence loss caused by *Pythium* at low temperatures, warm weather favored total damping-off, irrespective of the fungus acting. When temperature was low, postemergence damping-off was at a minimum.

High soil moisture favored *Pythium* while greatest *Rhizoctonia* damage occurred in dry soils. When both fungi were present, the common fluctuations in soil moisture did not greatly affect total damping-off. Warm weather, combined with very high relative humidity, favored rapid aerial growth of *Rhizoctonia*.

An understanding of the conditions favoring or inhibiting the activity of these fungi insures a logical application of control measures.

LITERATURE CITED

(1) Gifford, C. M.
1911. The damping off of coniferous seedlings. Vt. Agr. Expt. Sta. Bul. 157: [141]–171, illus.

(2) Hansen, T. S., Kenety, W. H., Wiggin, G. H., and Stakman, E. C.
1923. A study of the damping-off disease of coniferous seedlings. Minn. Agr. Expt. Sta. Tech. Bul. 15, 35 pp., illus.

(3) Hartley, C.
1921. Damping-off in forest nurseries. U. S. Dept. Agr. Bul. 934, 99 pp., illus.

(4) Jones, L. R.
1908. The damping off of coniferous seedlings. Vt. Agr. Expt. Sta. Ann. Rpt. (1906–1907) 20: 342–347.

(5) Roth, C.
1935. Untersuchungen über den Wurzelbrand der Fichte (Picea excelsa Link). Phytopath. Ztschr. 8: 1–110, illus.

(6) Roth, L. F., and Riker, A. J.
1943. Life history and distribution of Pythium and Rhizoctonia in relation to damping-off of red pine seedlings. Jour. Agr. Res. 67: 129–148, illus.

(7) ——— and Riker, A. J.
1943. Influence of temperature, moisture, and soil reaction on the damping-off of red pine seedlings by Pythium and Rhizoctonia. Jour. Agr. Res. 67: 273–293, illus.

(8) Wilde, S. A.
1934. Soil reaction in relation to forestry and its determination by simple tests. Jour. Forestry 32: 411–418, illus.

(9) ——— and White, D. P.
1939. Damping-off as a factor in the natural distribution of pine species. Phytopathology 29: 367–369, illus.

SOME CHEMICAL RELATIONS IN THE SUGAR BEET DURING PHASES OF ITS DEVELOPMENT [1]

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INTRODUCTION

Investigations at the Arizona Agricultural Experiment Station on sugar beets (*Beta vulgaris* L.) grown for seed have dealt principally with the physiology of seedstalk induction. The primary objective has been to determine the effect of temperature relations as modified by cultural practices upon vegetative and reproductive development. As a part of the general problem a chemical study of the entire plant was made throughout its life cycle under the climatic conditions which prevail in the Salt River Valley of Arizona and which are typical of the areas of the Southwest where sugar beets are grown for seed by the overwintering method (*6*).[3]

Winter temperatures in many beet-seed-producing areas are near the critical temperature that determines vegetative or reproductive development. In the problem to be discussed in this paper temperature is the chief variable. The lack of specific information on seed-producing plants under field conditions prompted this study of the principal labile carbohydrate and nitrogen fractions in top and root of the sugar beet at intervals during plant development for two successive seasons. Certain chemical relationships revealed by the data are emphasized herein because they suggest interesting phases of the physiology of the beet plant and, possibly, something of the general problem involved in phasic development.

The commercial sugar beet is a biennial, and after a dormant winter period it begins growth anew and forms seedstalk, flowers, and fruit. However, under constant warm greenhouse conditions it will continue vegetative growth indefinitely. Suitable conditions of cool temperature must be experienced by the plant to induce the reproductive phase. Sugar-beet plants differ greatly in their tendency to bolt, that is, to form seedstalks. Plants that respond to relatively slight exposure to cold are usually referred to as easy or fast bolters, while those that require relatively great exposure to cool temperature are hard or slow bolters. Plants grown for seed are not thinned, in contrast to the practice of thinning beets grown for sugar production. Early plantings made in August have a longer period of vigorous growth during the autumn than later plantings, and the larger foliar cover shades the soil more completely and reduces the surface soil temperature. Thin stands of plants reduce this cooling effect.

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[2] Valuable advice given by Dr. Eubanks Carsner during the course of these experiments is gratefully acknowledged.
[3] Italic numbers in parentheses refer to Literature Cited, p. 445.

Both vegetative and reproductive sugar-beet plants produce large storage roots. Typical differences in their shape, size, and sugar content have been discussed by Esau (*2*). In this study the roots of bolting plants were smaller and attained a higher sucrose concentration than the roots of typically vegetative plants, as grown for sugar, but because of their greater size, the vegetative plants contained greater absolute amounts of sucrose per plant. The leaf area was approximately equal in vegetative and reproductive plants of the same age.

The general effect of temperature upon reproduction in the sugar beet is well known. Chroboczek (*1*) and Roberts and Struckmeyer (*10*) found it to be a long-day, cool temperature plant. Owen, Carsner, and Stout (*7*) showed that the relative effects of temperature and day length vary with the genetic constitution of the plant. For biennial beets they found that the temperature effect was predominant, but also found evidence of the production of flower-inducing substances by the plant. Information on the chemical behavior of sugar beets has been provided by the work of Pultz (*9*) at St. George, Utah, on a variety of easy-bolting beets grown under conditions of temperature favorable to bolting, in which the chief variable was the nitrogen supply of the soil. It was shown by Pultz that sucrose and nitrogen were withdrawn from the root during seedstalk development. When nitrogen was limiting, sucrose accumulated, flowering was suppressed, and the seed yield was lowered.

EXPERIMENTAL DETAILS

Sugar beets were grown in plots near Mesa, Ariz., within the seed-producing area of the Salt River Valley. In the first year's experiments six varieties [4] of sugar beets varying in characteristics with respect to disease resistance and bolting tendency were grown in duplicate plots of approximately 0.1 acre per variety for each of three planting dates (August 16, September 17, and October 2). The plants were grown on soil judged from past records to be of high and uniform fertility. Ten tons of barnyard manure per acre was applied before the soil was prepared. Ammonium phosphate was applied at the time of seeding. Seed was sown at the rate of 18 pounds per acre in rows 20 inches apart on two-row beds centered 24 inches apart. Additional nitrate was added March 7. It is believed that no nutritional factor was limiting, except possibly nitrogen for a short period during the first winter in the August and September plantings. Differences in plant responses are assumed to reflect the effects of temperature. Cultural practices incident to date of planting, extent of shading, spacing of plants, and irrigation provided a differential of temperature whereby the soil at the crown of the beets of the August planting was several degrees cooler than that of the October planting during several hours each day for most of the growing season, as shown by thermograph records (fig. 1). A sample for a chemical test was a composite of all varieties for each planting date. Determinations for reducing sugars, total sugars, amino nitrogen, total nitrogen, nitrate nitrogen, and amide nitrogen were made November 25, December 29, March 2, April 13, and June 3. Soil nitrate was

[4] The varieties grown were U. S. 12, U. S. 14, U. S. 33, U. S. 217, Great Western, and Rabbethge & Giesecke (old type).

determined for each foot increment to a depth of 5 feet for each plot on each of these dates. The soil was Laveen loam, characterized as follows: [5]

Total soluble salts, 1,200 p. p. m. of dry soil
pH at moisture equivalent, 7.7
at 1:10 dilution, 8.8
Neubauer values:
23 mg. K per 100 gm. of soil
5.2 mg. PO_4 per 100 gm. of soil

Experiments for the second season were limited to 1 slow-bolting variety planted in duplicate plots of approximately 0.3 acre per plot for each date of planting. Five of the 32 rows in 1 plot of each planting date were thinned to 1 plant per foot to produce a slight difference in soil temperature between plants of the same date of planting, but otherwise environmental conditions were the same. Chemical de-

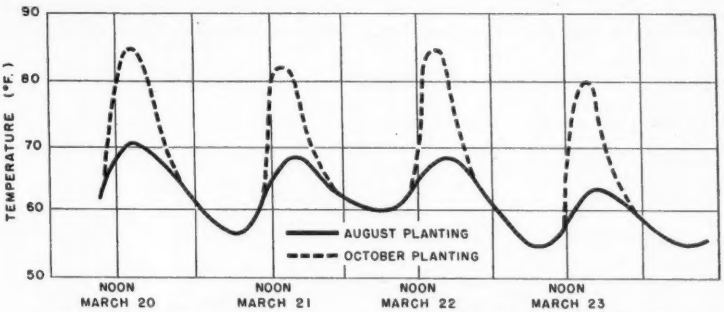

FIGURE 1.—Soil temperature at crown of beets during March 1939.

terminations were made upon both thinned and unthinned plants of the August and October plantings and also upon the unthinned September plants. Thus each season 3 plantings were made, designed to produce different degrees of thermal induction of the reproductive phase. The relatively cold winter of the first season favored this induction and a high seed yield except in plants at the extreme south end of the plots where the soil was subjected to little shading and no induction occurred. These latter plants were used as typical nonbolters. In the second season three degrees of thermal induction were provided by date of planting as before, and in addition a more precise study of slight temperature differences was provided by spacing of the plants. The chance of variability as a factor was reduced by limiting the study to 1 variety. Average temperature for the second season was less favorable for induction of the reproductive phase, thus placing all plantings of that season very near the critical temperature relationship.

CHEMICAL METHODS

A relatively large amount of material was gathered from each plot and rushed to the laboratory under cool, moist conditions where representative samples of sap and tissue were prepared and refrigerated. Amino acids were determined at once on the sap by the

[5] Data furnished by Dr. T. F. Buehrer of the Arizona Agricultural Experiment Station.

Van Slyke method (*11*), amides were promptly hydrolyzed and their determination completed shortly thereafter by the Vickery-Pucher method (*12*), sugars were determined from samples of the expressed sap by the ceric sulphate method described by Hassid (*4*), and nitrate by the Devarda method. Total nitrogen was found by the Kjeldahl method on finely ground tissue in which the nitrate had been reduced by iron (*8*). Soil nitrate was ascertained by the phenoldisulfonic method.

RESULTS

Solutes are reported in terms of concentration on the assumption that concentration has greater physiological significance than dry-weight relations. Figure 2 illustrates the general behavior of reducing sugars as shown by the seed-producing plants for 1938–39. In the top tissue there was an increase in the concentration during early development, followed by a decline during seed development. The root tissue was characterized by a very low concentration of reducing

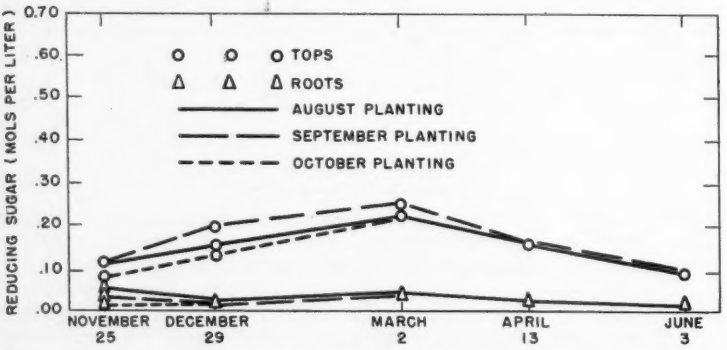

FIGURE 2.—Reducing sugars in sugar beets during development, 1938–39.

sugars throughout the season. A marked difference existed between root and top concentration, but this difference was characteristic of both vegetative and reproductive plants. The second season showed the same general trend except that no significant decline in concentration of reducing sugar occurred in the top tissue during the reproductive stage. The seed yield for the second season was quite low.

The concentration of sucrose in plants for 1938–39 is shown in figure 3. The early plantings (August and September) rapidly built up the sucrose concentration to high levels until bolting time, after which it rapidly declined. These plants produced heavy seed yields. The October planting maintained a lower sucrose level during its vegetative development, but continued to increase the concentration even during the bolting period, and markedly so during the period of seed development to the extent that on June 3 the late (October) planting had a decidedly higher concentration of sucrose than the early plantings. The absolute amount of sucrose per plant was much less in the late than in the early plantings. The October planting yielded less than one-half the seed produced by either of the earlier plantings.

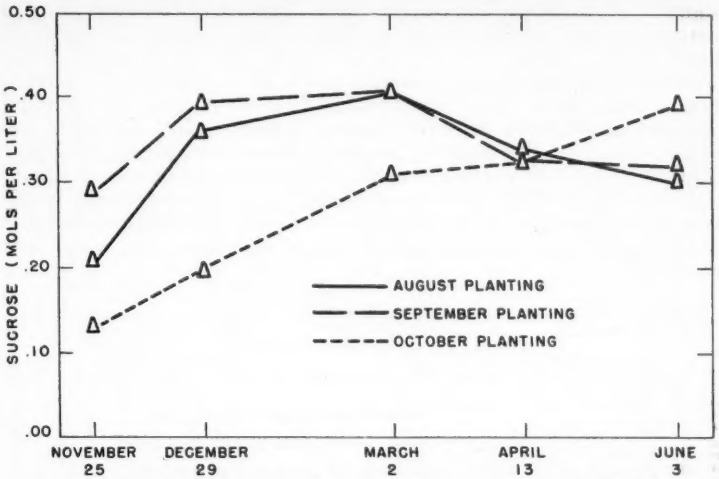

FIGURE 3.—Sucrose in sugar-beet roots during development, 1938–39.

Figure 4 indicates the trends of sucrose concentration observed during the season 1939–40. August and October plantings each have parallel experiments, namely, with thinned and unthinned plants. All plantings show a continuous increase of sucrose concentration in

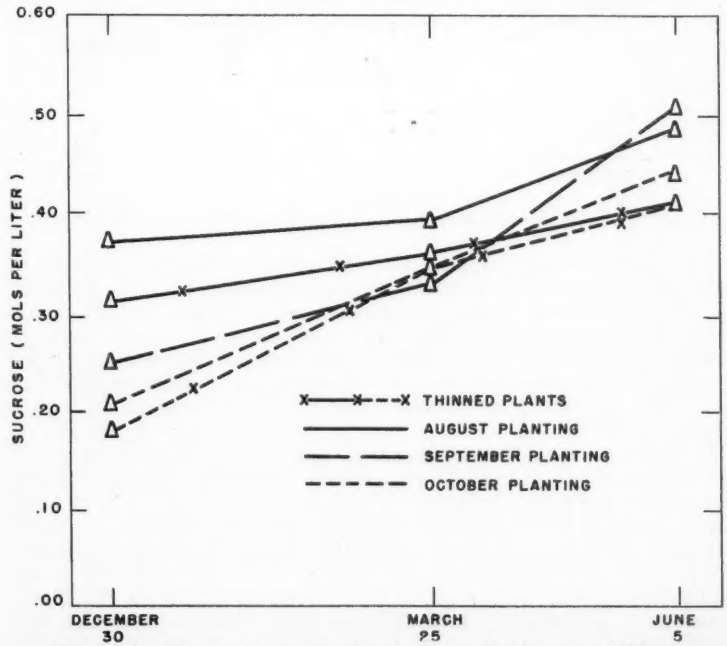

FIGURE 4.—Sucrose in sugar-beet roots during development, 1939–40.

the root tissue throughout the season. The early plantings (August and September) again showed the higher concentration in the early stage of development, but by June 5 the late planting (October) approached the same level of sucrose as the earlier plantings. During early development the thinned plants in both August and October plantings maintained a lower sucrose concentration than the unthinned plants. The thinned plants of these two widely divergent planting dates both showed a decline in the rate of sucrose accumulation during the bolting season and both attained approximately the same level of sucrose concentration by June 5. The thinned October planting produced no seed and all other plantings gave very poor seed yields.

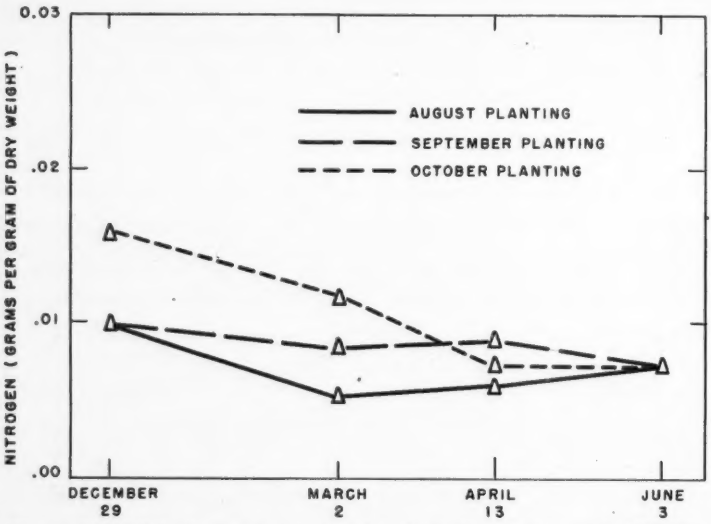

FIGURE 5.—Total nitrogen in sugar-beet roots during development, 1938–39.

The seasonal trend of total nitrogen in root tissue for 1938–39 is indicated in figure 5. A decline in the percentage of total nitrogen for all reproductive plants is characteristic. When data for these plants are compared with those for nonbolting plants (table 1) it appears that relatively large absolute amounts of nitrogen have disappeared from the roots during reproductive development. The major loss apparently takes place about the time of seedstalk development.

In figure 6 is shown the trend of total nitrogen in August and October plantings for the following season (1939–40). The unthinned plants show a marked decline during the bolting period. The thinned plants show a distinctly different behavior with respect to total nitrogen. The thinned August planting bolted and produced some seed; the thinned October planting produced no seed. Both thinned plantings were relatively high in total nitrogen while both unthinned plantings produced more seed and lost total nitrogen from the root tissue.

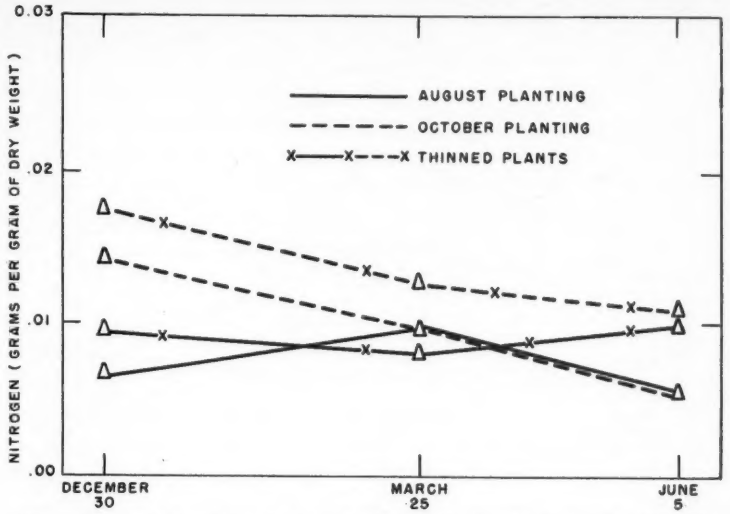

FIGURE 6.—Total nitrogen in sugar-beet roots during development, 1939–40.

TABLE 1.—*Comparison of carbohydrate and nitrogen fractions in bolting and non-bolting sugar-beet plants at harvesttime*

Fraction	Tissue	Planting date			
		Bolters		Nonbolters	
		Aug. 16	Sept. 7	Oct. 2	Sept. 7
Reducing sugars.....mols per liter	Tops	0.071	0.078	0.078	0.055
	Roots	.006	.007	.006	.009
Sucrose.....do	Tops	.000	.006	.005	.002
	Roots	.324	.341	.387	.315
Nitrate nitrogen.....do	Tops	.0083	.0087	.0043	.0478
	Roots	.0039	.0071	.0066	.0636
Amide nitrogen.....do	Tops	.0036	.0019	.0073	.0062
	Roots	.0013	.0013	.0013	.0096
Amino nitrogen.....do	Tops	.0220	.0173	.0188	.0519
	Roots	.0162	.0239	.0298	.1163
Total nitrogen per gram of dry weight grams	Tops	.0441	.0273	.0316	.0318
	Roots	.0068	.0070	.0070	.0155

Nitrate nitrogen represents from 3 to 11 percent of the total nitrogen found in the plant. The nitrate relationships are illustrated in figures 7, 8, 9, and 10. The trend of nitrate nitrogen in the tops of the 1938–39 plants, which is shown in figure 7, is characterized by a marked reduction in concentration during the reproductive phase. A similar trend in the root tissue is indicated in figure 8. Figure 9 shows the behavior of the plants in the parallel experiments in the August planting of the 1939–40 season. The unthinned plants show the same decrease in nitrate concentration noted before, but the thinned plants show little change in concentration in either top or root tissue during the period of flowering and seed development. Similar trends are apparent in the October planting (fig. 10). In all cases except the October planting of 1938–39 the unthinned seed-producing plants

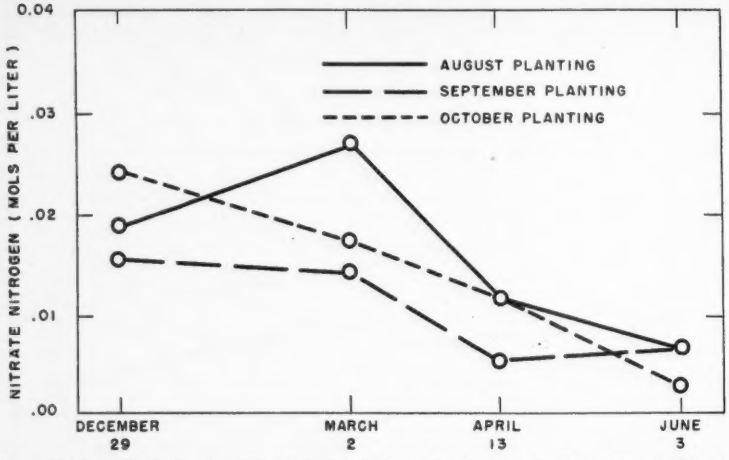

FIGURE 7.—Nitrate nitrogen in sugar-beet tops during development, 1938–39.

developed a nitrate relationship such that the concentration was relatively much greater in the tops than in the roots. In the parallel experiments the thinned plants either reversed this relationship or showed a strong tendency to do so. The concentration of nitrate in the tissues of the plant was always manyfold greater than the concentration in the soil (figs. 9 and 10).

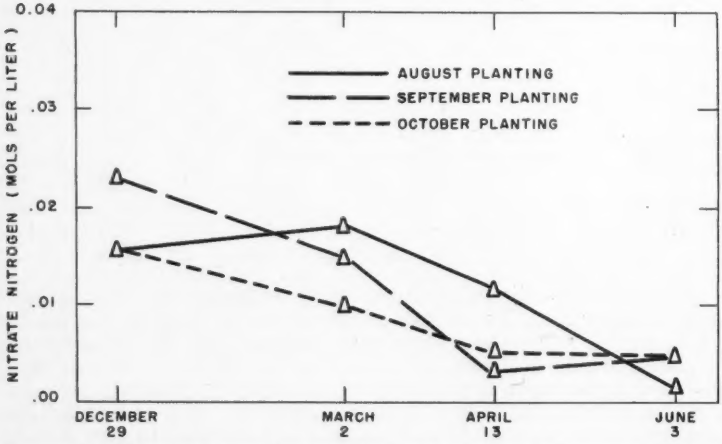

FIGURE 8.—Nitrate nitrogen in sugar-beet roots during development, 1938–39.

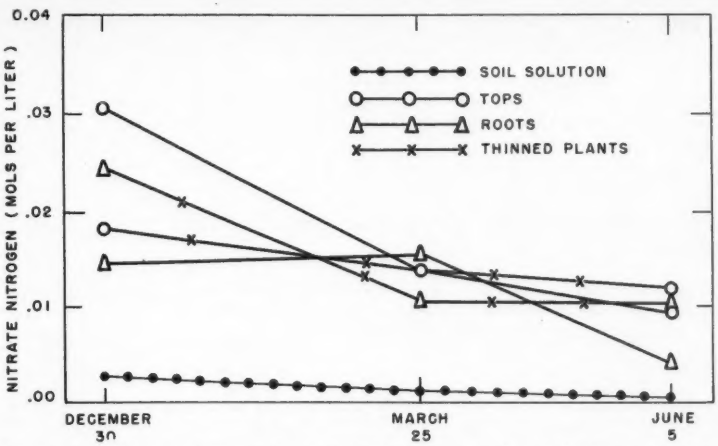

FIGURE 9.—Nitrate nitrogen in sugar beets (August planting) during development, 1939–40.

The dominance of the vegetative or reproductive phase of development during the season is illustrated by the relative weight relations between top and root for the first season's plantings as shown in table 2. The degree of successful reproduction attained by the

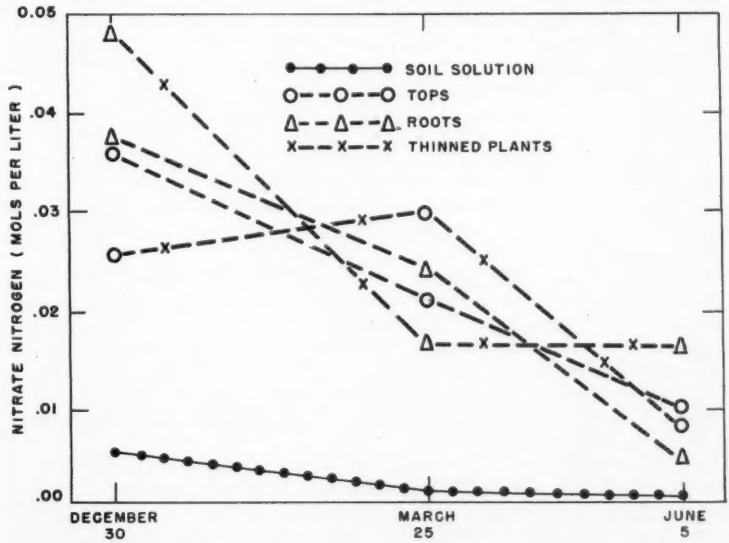

FIGURE 10.—Nitrate nitrogen in sugar beets (October planting), during development, 1939–40.

TABLE 2.—*Top-root weight ratios of sugar-beet plants grown for seed, 1938–39*

Planting date	Date of sampling			
	Nov. 25	Dec. 29	Mar. 2	Apr. 13
Aug. 16	1.63	1.76	1.19	1.68
Sept. 7	4.38	2.97	1.81	2.76
Oct. 2	6.85	3.47	2.10	1.57

various plantings may be estimated from the seed yields given in table 3.

TABLE 3.—*Seed yields*[1] *from a variety of sugar beets low in bolting tendency*

Planting date	Yield per acre	Planting date	Yield per acre
1938	*Pounds*	*1939*	*Pounds*
Aug. 16	2,168	Aug. 23	580
Sept. 7	2,545	Sept. 16	321
Oct. 2	917	Oct. 7	179

[1] No records made of yields from thinned plots; no seed produced by thinned plot of Oct. 7, 1939.

DISCUSSION

Both the vegetative and reproductive phases of development in the sugar beet are favored by an abundant supply of nitrogen (*9*). Since the sugar beet is a long-day plant the induction of the reproductive phase should cause neither check in growth nor accumulation of reserves as has been reported for short-day plants (*5*). Therefore, characteristic trends in chemical relations shown by the data on sugar beets appear to have more than ordinary significance. Trends which appear important here are the carbohydrate, total nitrogen, and nitrate nitrogen relationships during the vegetative and reproductive phases of the plant. The need for discernment of various stages in the reproductive phase brought about by either photo induction or thermal induction has been stressed by Hamner (*3*). For the sugar-beet plant attention may be focused upon those plants that are strictly vegetative, those that bolt but produce little seed, and those that produce heavy seed yields.

Reducing sugars throughout the plant were generally of the same order in corresponding parts of plants of the same age and their concentration and movement did not seem to be a limiting factor except in the October planting for 1938–39 where the low concentration in the tops conceivably could have been responsible for low seed yields. The decline in reducing sugars wherever heavy seed production was in process suggests that reducing sugars constitute an important step in these transformations.

Sucrose appears to be the principal source of mobile carbohydrates, supplemented by the daily contribution by photosynthesis. Plants that produce heavy seed yields draw heavily upon the sucrose in the root. Such utilization involves a hydrolytic process which did not appear to be limiting except, possibly, in the case noted above. Plants that bolt but make poor seed yields continue to increase the sucrose concentration in the root tissue during the bolting period. Plants of the same age that remain strictly vegetative likewise con-

tinue to accumulate sucrose. The tendency to store or utilize sucrose apparently becomes a measure of vegetative or reproductive tendencies in the beet plant.

During the reproductive phase the percentage of total nitrogen decreased more rapidly than the rate of growth of the root, about 50 percent of the nitrogen actually disappearing from the root. In the vegetative plant of the same age the percentage of total nitrogen increased during the same period. Since only a small percentage of the nitrogen was soluble at any given time a hydrolytic process was involved in the reproductive phase. The tendency to export nitrogen from the root may be considered a reproductive characteristic of the sugar beet.

So far as may be judged from the results of this study, the nitrate nitrogen appears to be the most mobile nitrogen fraction. At all times the nitrate concentration was manyfold greater throughout the plant than in the soil solution. The equilibrium concentration (or steady state) near seed harvesttime was markedly different in plants of the same age but in different stages of development. In strictly vegetative plants the nitrate concentration was severalfold greater than in reproductive plants. It had an intermediate value in wealky reproductive plants. In vegetative plants the concentration in the root exceeded the concentration in the top as if the nitrate distribution depended upon a concentration gradient from the root. In typically reproductive plants the nitrate equilibrium maintained a higher concentration in the top as if the nitrate were transported through the plant by the utilization of energy, as has been postulated for other ions. The tendency for nitrate to accumulate in the tops of reproductive plants has been observed also by Dr. J. M. Fife [6] in four successive seasons. Unthinned plants from the parallel experiments of the second season followed the pattern for reproductive plants, while the thinned plants, subjected to slightly higher soil temperatures showed the nitrate relationship characteristic of vegetative plants. The low-yielding plants (October) of 1938–39 that showed strong vegetative characteristics with regard to sucrose accumulation and top-root weight ratio also had a nitrate relationship characteristic of vegetative plants.

In those cases where the nitrogen supply and temperature were both favorable the plants utilized nitrogen and sucrose from the root, a finding which is in agreement with previously reported observations of Pultz (*9*). When the temperature relation was unfavorable for reproduction this fact was reflected in the chemical relations within the plant and nitrogen was not used freely for flower and seed even in the presence of an ample supply of nitrogen in the plant tissue. Seed yields and temperature records for these experiments fully support the conclusions of Owen, Carsner, and Stout (*7*) on the relation of temperature to bolting. Differences in certain chemical relations observed in plants that experienced different temperature exposures may be interpreted as supporting the concept of the induction of substances essential to the reproductive phase through temperature relations. However, the nature of the special agents responsible for the observed chemical relations is conjectural.

The foregoing observations show that the loss of sucrose from the root is roughly proportional to the seed production, the loss of nitrogen

[6] Correspondence.

is greatest during seedstalk formation, and the most striking changes in nitrate concentration and top-root nitrate ratio occur during the period of seed maturation. There appears to be fairly good evidence for a correlation of the storage of sucrose in the root, high total nitrogen, high nitrate concentration throughout the plant, a top-root nitrate ratio less than unity, and a decreasing top-root weight ratio with the vegetative phase. On the other hand, the loss of sucrose and nitrogen from the root, a declining nitrate concentration, a high top-root nitrate ratio, and a high top-root weight ratio are charcateristic of the reproductive phase. The change in equilibrium for any of these systems appears to be associated with a certain stage of development. The extent to which these relationships between development and chemical composition are causal or resultant is debatable. The more important consideration is the agency that directs the chemical reactions toward one or the other phase of development.

Ordinarily each of the important processes that have been discussed is considered to be controlled rigidly by one or more enzyme systems. The agents necessary to the initiation of these processes are produced by this plant under favorable conditions of temperature. The temperature requirements for their production differ significantly and the degree to which any of the chemical relationships is attained in the plant depends upon thermal relationships. It is not clear whether a group of enzyme systems acting independently performs the role sometimes attributed to a flowering hormone or whether the operation of these systems depends upon the activity of one or more hormone-like substances. The need for a better understanding of enzyme systems in plant development is evident. The data indicate that a number of processes, such as the hydrolyses of sucrose and insoluble forms of nitrogen, the special distribution of nitrate, reduction of nitrate and syntheses of certain compounds, are involved in reproduction in the sugar beet and that the coordination of all these processes is essential to successful reproduction. It appears that a consideration of the larger chemical fractions as found in suitable plants grown under appropriate conditions will contribute much toward the analysis of the problems of phasic development.

SUMMARY

Sugar beets were grown for seed in the Salt River Valley of Arizona under favorable nutritional conditions. Temperature relations were varied through cultural practices. Chemical determinations were made on important carbohydrate and nitrogen fractions in tops and roots throughout the vegetative and reproductive phases for two successive seasons.

Comparison of these chemical fractions, as found in strongly vegetative plants and in plants subjected to various degrees of induction to the reproductive phase, has been made. Seed yield was used as a criterion of successful reproduction.

Vegetative plants stored sucrose and nitrogen in the roots. Nitrate attained a relatively high concentration throughout the plant, with the root concentration in excess of the top.

High seed-yielding plants utilized sucrose and total nitrogen stored in the root. Nitrate showed marked reduction in concentration during the reproductive phase, characterized by a higher concentration in top than root.

Low seed-yielding plants utilized less than the daily supply of sugars provided by photosynthesis and stored sucrose during the bolting period. Nitrogen relations within the plant reflected the degree of induction to the reproductive phase brought about by temperature.

Some possible relationships between chemical composition and phasic development have been discussed.

LITERATURE CITED

(1) Chroboczek, E.
1934. A study of some ecological factors influencing seed-stalk development in beets (beta vulgaris l.) N. Y. (Cornell) Agr. Expt. Sta. Mem. 154, 84 pp., illus.

(2) Esau, K.
1934. Bolting in sugar beets. Facts About Sugar 29: 155–158, illus.

(3) Hamner, K. C.
1938. Correlative effects of environmental factors on photoperiodism. Bot. Gaz. 99: 615–629.

(4) Hassid, W. Z.
1937. Determination of sugars in plants by oxidation with ferricyanide and ceric sulfate titration. Indus. and Engin. Chem. (Analyt. Ed.) 9: 228–229.

(5) Murneek, A. E.
1937. Biochemical studies of photoperiodism in plants. Mo. Agr. Expt. Sta. Res. Bul. 268, 84 pp., illus.

(6) Overpeck, J. C., and Elcock, H. A.
1931. Methods of seed production from sugar beets overwintered in the field. U. S. Dept. Agr. Cir. 153, 22 pp., illus.

(7) Owen, F. V., Carsner, E., and Stout, M.
1940. Photothermal induction of flowering in sugar beets. Jour. Agr. Res. 61: 101–124, illus.

(8) Pucher, G. W., Leavenworth, C. S., and Vickery, H. B.
1930. Determination of total nitrogen of plant extracts in presence of nitrates. Indus. and Engin. Chem. (Analyt. Ed.) 2: 191–193.

(9) Pultz, L. M.
1937. Relation of nitrogen to yield of sugar-beet seed and to accompanying changes in composition of the roots. Jour. Agr. Res. 54: 639–654, illus.

(10) Roberts, R. H., and Struckmeyer, B. E.
1938. The effects of temperature and other environmental factors upon the photoperiodic responses of some of the higher plants. Jour. Agr. Res. 56: 633–677, illus.

(11) Van Slyke, D. D.
1913. The gasometric determination of aliphatic amino groups in minute quantities, Jour. Biol. Chem. 16: 121–124.

(12) Vickery, H. B., and Pucher, G. W.
1931. A source of error in the determination of amide nitrogen in plant extracts. Jour. Biol. Chem. 90: 179–188.

INHERITANCE OF SIZE IN SINGLE-COMB WHITE LEGHORNS [1]

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INTRODUCTION

Investigators who have studied the inheritance of body size have employed a variety of methods, the most common of which involve the crossing of animals of divergent sizes. The mean size and variability of the F_1, subsequent generations, and backcrosses have led most workers to conclude that body size is under polygenic control. Usually animals of different breeds or varieties of a given species or even animals of different species have been used in such studies, since the existing interbreed and interspecific variability provides a wide range of characteristic body sizes. This is especially true of the domestic animals in which generations of artificial selection have resulted in the establishment of breeds differing in size by several hundred percent.

Instances of crosses between breeds of poultry of such extreme divergence are furnished by the studies of Punnett and Bailey (*18*),[2] Jull and Quinn (*8*), and Maw (*16*), all of whom used Bantams as one of the parents. As noted, inheritance on a polygenic basis was indicated in these cases, as well as in crosses between breeds less dissimilar in size, including those made by authors tending to interpret the F_1 and other segregations on the assumption of a limited number of genes (cf. the two-gene hypothesis of Waters (*22*) or the "four or more" pairs of genes suggested by Quisenberry, Roberts, and Card (*19*). Interpretation on a polygenic basis also appeared to satisfy the results of studies conducted on crosses in other species than chickens, e. g., ducks (*17*, *4*), pigeons (*23*), and most of the commonly studied laboratory mammals. (See Lerner (*9*) for a review.)

The variation in mature size within breeds has been studied far less intensively. In chickens Lerner (*10*) ,using shank length as a criterion of size, found two strains of Single-Comb White Leghorns to exhibit significant differences, with the F_1 and backcrosses intermediate in size. Earlier Dunn (*3*) reported similar differences in the length of the long bones between inbred families of the same breed. Of two crosses between such families one F_1 was intermediate and the other exceeded both parents.

Other investigations on the genetic basis of size differences within breeds include a limited number of statistical studies on Jersey cattle and swine. In the former Gowen (*7*) noted that some 60 percent of the variance in size was hereditary. In swine the genetic portion of the variance was found to rise from an insignificant figure at birth (*14*) to 18 percent at weaning time (*2*), and to 30 to 40+ percent at 180

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[2] Italic numbers in parentheses refer to Literature Cited, p. 456.

days of age (*24*). The increase of the genetic portion of the variance with age reflects the existence of significant nongenetic effects of nutrition during the embryonic and the nursing stages of mammals (at weaning time 40 percent of the total variance was due to environment common to litter mates). Such effects are likely to be of much smaller importance at comparable ages of birds.

All in all, it may be seen from the above review of literature that the information on the extent of the hereditary control of adult size within breeds is very limited. The present study was designed to throw some light on this subject, with the Leghorn breed of chickens used as experimental material.

MATERIAL AND METHODS

Body size may be expressed in terms of body weight or in terms of some skeletal measurement. The latter is preferable since body weight is subject to a great deal of fluctuation due to nutritional state, condition of the ovary, and other factors of internal and external environment. Mature length of shank, a measurement bearing a close relation to the actual length of the tarsometatarsus ($r = 0.968 \pm 0.007$ with a standard error of estimate of 0.028 cm. (*1*) has been found to be of use in expressing size differences. Its correlation with body weight is of the magnitude of 0.66 (*10*), so that not all of the variation in body weight is accounted for by shank-length differences between birds. For the purposes of the present study mature shank length is used as a criterion of size but with the understanding that it does not necessarily represent the same genetic differentials that would be manifested by the variation of body weight.

Measurements of shank length were made on mature females from the University of California flock with the device described by Bumester and Lerner (*1*). The production-bred strain of Leghorns with which the experiment originated consisted of approximately 450 to 650 pullets hatched annually in March and April. These were selected for high production and viability since 1933 with some introduction of stock from private breeders. Of particular importance in connection with this experiment is the extensive use made in breeding of the offspring of 3 males and 1 female from the strain developed by the Kimber Breeding Farms at Niles, Calif. An earlier report (*10*) gave detailed information on the differences in size between the Kimber strain and the University of California flock. The mature females of the Kimber strain averaged 10.28 cm. in shank length as compared with a mean of 9.59 cm. for the University flock.

In 1938 when the present experiment was started, a considerable proportion of the University flock had one or more of the four Kimber birds represented in their pedigrees. In December of that year the first measurements relating to this study were made. All full-sister families of five or more pullets originating from sires with a minimum of three such families were measured. In subsequent years the same procedure was followed, but the bases of selection of breeding birds to perpetuate the production line did not take these measurements into consideration. The production line can thus be considered as a control population, random-bred with respect to size. The exception to this statement lies in the fact that the foundation stock for the large size line was made ineligible to serve as breeders for the production

line. In this manner a minor degree of selection against large size was exercised in these quasi controls.

The size line of 1939 originated from two males and eight dams. In the following years several dams not previously represented in the size line were introduced from the production line (four in 1940, and two in 1941). In the size selection all of the female offspring alive in December of their year of hatch were measured irrespective of the number of sisters in the family.

The number of birds measured in each year in each line and the mean shank measurements appear in table 1 under the heading

TABLE 1.—*Mean shank length for birds measured in each year in each line*

Year	Total population				Restricted population			
	Size line		Production line		Size line		Production line	
	Birds measured	Mean shank length	Birds measured	Mean shank length	Birds measured	Mean shank length	Birds measured	Mean shank length
	Number	*Centimeters*	*Number*	*Centimeters*	*Number*	*Centimeters*	*Number*	*Centimeters*
1938			368	9.69			368	9.69
1939	84	9.92	274	9.62	84	9.92	240	9.65
1940	83	9.94	137	9.55	59	9.89	121	9.59
1941	106	10.20	346	9.37	61	10.18	346	9.37
1942	119	10.29	260	9.46	86	10.30	256	9.46

"Total population." For the purposes of statistical analyses in a subsequent section those families which answered the requirements of numbers as outlined for the production line were selected. The mean shank measurements for these birds also appear in table 1 under the heading "Restricted population." The discrepancies between the number of birds in the two populations of the production line are due to the fact that in some cases measurements on all members of a family were not obtained, thus reducing the size of the family below the minimum number set. Failure to obtain measurements on these birds was usually due to bumblefoot, an abscess on the ball of the foot, which prevented the application of the measuring device.

The selection of breeding birds in the size line was based on progeny- and sister-test data, and, within families of full sisters, on the phenotype. Some effort was made to maintain in the size line the high egg-production characters of the production line, with particular emphasis on early sexual maturity. In order to maintain the economic qualities of the size line late-maturing birds (over 200 days of age at their first egg) were discriminated against in the selection of breeders. In spite of this the 1942 size line matured significantly later than the corresponding production line by 10.7 ± 3.0 days (176.9 as against 166.2 days).

Males were selected entirely on the basis of progeny and sister tests. It has not been economically possible to keep all the males till maturity, and the information on the relation of early to mature shank length in these lines has not been sufficient to base the selection of males on their shank length at 6 weeks of age (the time when most males are culled in this flock).

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It should be noted that the small increase in the mean shank length of the fourth selected generation over that of the third (0.09 cm.) does not necessarily indicate an approach to a plateau. It so happens that one of the three sires used in 1942 had progeny averaging lower than the mean of the 1941 population (10.06 vs. 10.20 cm.). The number of offspring from the other two sires (averaging 10.36 and 10.43 cm. respectively) was not sufficiently large to compensate entirely for the choice of this one male.

As noted previously, the selection of breeders in the production line was independent of the measurements made. The amount of inbreeding in this line did not show any rapid increase in the experimental years. As an indication of this, the average coefficients of inbreeding of the sires of the restricted population were for the years 1938 to 1942, respectively, 4.13, 7.26, 5.94, 5.25, and 2.93 percent. Similar figures for the sires of the size line (restricted poulation for the years 1939 to 1942) were 3.52, 7.55, 2.35, and 11.81 percent. A better indication of the increasing homozygosity of the size line is given by the inbreeding coefficients of the extreme variant families of sisters (with the longest shanks) in that line for each year from 1939 to 1942, which were respectively 3.13, 4.69, 7.47, and 15.95 percent.

RESULTS OF SELECTION

As table 1 shows, the four generations of selection have undoubtedly been successful in increasing the shank length of the size line and differentiating it from the production line.

There are at least two possible factors responsible for lack of even greater progress: (1) The small scale of breeding operations, and (2) the damping effect on selection for size exercised by the discrimination against late-maturing birds. A third possibility, although of much more speculative nature, is that the elimination of birds with bumble-foot had a similar effect, if there exists an association between size of bird and incidence of this defect.

There is no indication that a plateau has been reached in the size line. It should, however, be noted that, as shown in table 2, the range of individual values in the size line has not been extended upward from that found in the production line. Furthermore, the parent flock of the Kimber birds which were introduced into the University flock averaged, as has already been pointed out, 10.28 cm., a value nearly identical with the mean of the fourth selected generation. Although the four Kimber birds figure as prominently in the pedigrees of the current production flock as they do in the size line, it is probable that up to this point all that selection has accomplished is to reconstitute the original genotype for size of the Kimber flock. In contrast to this, Goodale (*5*, *6*) was able by similar methods of selection to increase the size of mice considerably beyond that of the original range in his foundation stock.

There are two important differences, however, between Goodale's experimental project and the one reported here. In the first place, his size determinations were made on growing animals rather than on adults. It is possible then that some of the increase in size observed by him (but not all, as is obvious from his data) was due to a more rapid earlier growth rate. Difference in growth pattern independent of mature size are known to exist. Thus Lerner and Asmundson (*12*)

described two strains of Leghorns equal in adult body weight but differing in weight during the early stages of growth. So far as the present data are concerned, growth-pattern differences are of no consequence, since previously gathered material on this stock (*11*) indicates that growth in length of the shank ceases considerably before the age at which the present measurements were made.

TABLE 2.—*Distribution of individual birds according to shank length by year and line (total population)*

Shank length (centimeters)	Individual birds by year and line								
	1938	1939		1940		1941		1942	
	Production line	Production line	Size line	Production line	Size line	Production line	Size line	Production line	Size line
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
8.1–8.3						1			
8.4–8.6		2				11		2	
8.7–8.9	5	18	1	5		33		16	
9.0–9.2	41	35	3	28	3	91		61	1
9.3–9.5	95	62	15	39	9	106	4	83	5
9.6–9.8	115	71	15	37	25	68	16	56	20
9.9–10.1	63	58	26	18	16	21	27	27	20
10.2–10.4	35	18	15	7	22	13	30	12	28
10.5–10.7	11	7	5	2	7	2	19	1	23
10.8–11.0	3	2	4	1	1		8	1	15
11.1–11.3		1					2	1	7
Total	368	274	84	137	83	346	106	260	119

The second difference between this and Goodale's experiment lies in the considerably greater scale of Goodale's work with many more generations and animals represented (28,000 mice as against less than 500 pullets in the present size line). Whether or not transgression of the original range can be achieved in the size line by further selection remains to be seen. Under Mather's (*15*) interpretation of the effects of selection on polygenically controlled characters, two series of advances in the direction of selection are probable, the first as a result of recombination of whole chromosomes followed by a plateau, and the second trend upward as a result of recombination of genes within chromosomes. In the present material the advance has been continuous and the reserve of genetic variance in size has by no means been exhausted (see below). Hence, it is likely that even the first phase of the progression described by Mather has not yet been completed.

As an indication of the relation of shank length to body weight, it may be stated that the fourth selected generation weighed on an average at the time of measurement 1,903 gm., while the mean weight of the birds in the production line of that year was 1,666 gm.

RESULTS OF CROSSES BETWEEN LINES

Reciprocal crosses between the two lines were made in each of the last 3 years reported upon here. They were designed so that size-line females mated to a given production-line male were full sisters of the size-line male which in turn was mated to the full sisters of the above

production-line male. In this manner it was hoped to circumvent to some extent any possible effect of intraline interfamily variability on the results of the crosses. The mean shank length for each of the groups of reciprocal matings is given in table 3. It may be seen that

TABLE 3.—*Mean shank length of daughters resulting from reciprocal crosses between the production and size lines*

Year	Sire		Daughters from—			
			Size-line dams		Production-line dams	
	Band No.	Line of origin	Birds measured	Mean shank length	Birds measured	Mean shank length
			Number	*Centimeters*	*Number*	*Centimeters*
1940	R16	Size	28	9.90	28	9.66
	R11	Production	11	9.46	11	9.15
1940	R33	Size	26	9.87	21	9.13
	R25	Production	4	9.53	34	9.63
1941	S31	Size	22	10.02	41	9.67
	S2	Production	10	9.31	27	9.14
1942	T50	Size	38	10.06	25	9.34
	T13	Production	25	9.52	24	9.25

in spite of the design of these matings the results were somewhat erratic, possibly because of the relatively small number of birds involved. The differences between reciprocal crosses in the 3 years are not always in the same direction. The grand mean of the 50 hybrids out of size-line dams was 9.50 cm., while the 115 hybrids out of production-line dams averaged 9.47 cm. This suggests that neither major sex-linked genes nor maternal effect are involved in the size difference between the two lines.

The F_1 values appear to be closer to those for the smaller parent, but whether dominance or other types of nonadditive gene action is involved cannot be determined with the material on hand. The curious fact that the F_1 values in the different years do not seem to reflect the increasing difference between the corresponding parental generations may be laid to the small number of birds involved. Attempts to analyze the data of table 3 by Wright's (*25*) method of estimation of the sire's genotype did not lead to any more successful interpretation, and are not presented here.

ESTIMATES OF GENETIC VARIABILITY

The usual methods of estimating the portion of variance that is due to differences between genotypes of parents are based on the degree of resemblance between relatives. Lush (*13*) has listed several such procedures with particular emphasis on the one employing the intrasire regression of offspring on dam. In the material on hand this method could not be applied without sacrificing a considerable part of the data on the production line since not all dams used in this line fell into the restricted population measured (see above), and hence were of unknown phenotypes. Furthermore, the separate contributions of the sire and the dam to the variance of their offspring cannot be evaluated by this method. Sex-linked genes, should any be

involved, would in the case of birds lead to underestimates of the degree of heritability, while maternal effects may on the other hand lead to overestimates. Nevertheless, when this method is applied to the restricted population of the size line, the degree of heritability is found to be equal to about 38 percent. This figure will be referred to again in a subsequent section.

Another of the methods described by Lush, which involves the comparison of the offspring with the average of the population from which their selected parents originated, requires for its efficient use selections in opposite directions and the continuity of ancestors within each line. Neither requirement is met in this material. Furthermore, neither the phenotypes of sires nor those of the population from which they originated are known.

To arrive at an estimate of the hereditary variance in the different populations, it is possible to isolate the genetic variance between families of full sisters without resorting to computation of correlations between relatives. The key to this method lies in the fact that all of the eggs from all of the birds bred each year were incubated in the same machines, the chicks reared in the same brooders, and the pullets housed together irrespective of ancestry. Under this system whatever environmental effects are present would operate entirely at random so far as the ancestry of the birds in any given hatch is concerned. In each year there were four hatches, but the representation of birds of different families in the different hatches was proportional. Hence, there is no reason to believe that members of a family or of a line shared an environment more common than the members of the flock as a whole. Under these circumstances it may be considered that the differences between the offspring of different matings attributable to differences between parents are genetic in nature. To separate further the genetic differences between lines from those within lines, the size line and the production line in each year need to be handled separately. In this manner the total variance of shank length of full-sister families in each generation may be separated into

1. Genetic variance between lines
2. Total variance within each line
   - (1) Genetic variance within each line
     - (a) Attributable to sires
     - (b) Attributable to dams
   - (2) Residual variance

The latter (residual variance) includes the nongenetic variance and that part of the genetic variance which is found within families of full sisters.

There are two possible objections to this method. The first of these arises from the fact that each dam was mated to only one sire within a given year. Hence the originally computed variance of offspring between sires includes also the differences due to the contributions of the dams. By calculating the variance of the offspring between dams within sires, the latter may be isolated. By subtracting the figure thus obtained from the original variance between sires the contribution of the sires may be assessed.

The second possible objection may be raised by questioning whether some of the differences between dams are due to nonhereditary maternal effects. Differences in nutritional content of eggs laid by differ-

ent dams may conceivably affect the growth of birds originating from them. In mammals such maternal influence is obviously more extreme (cf. the previously cited work on swine or the report of Walton and Hammond (*21*) on reciprocal crosses between Shetland ponies and Shire horses). In birds this effect is not likely to be great, since it is known, for instance, that egg size, and hence the limitation of the nutritional supply in the embryonic stage, bears no relation to chick weight beyond 2 to 4 weeks of age (*20*). However, even should there be some such effect, the genetic portion of the variance of shank length within lines between full-sister families could be estimated in the absence of sex-linkage by doubling the portion of the variance attributable to the sire on the assumption that the genetic contributions of the two parents are equal. This follows from the fact that the value for the sire is obtained by subtraction of the variance between dams within sires from the total interfamily variance, and thus is free from any possible maternal influence. As may be seen from the figures presented in table 6, twice the estimated contribution of the sire differs on an average by less than one-half of 1 percent from the estimated total interfamily genetic variance.

ANALYSIS OF VARIANCE OF SHANK LENGTH

Table 4 presents the complete analysis of variance for each year. The significant point in the analysis for the total population of each year is the great increase in the amount of variance due to differences between lines effected by selection. The increase is particularly notable in the last 2 years. As shown in the first line of table 5, in 1942 better than 40 percent of the total variance is attributable to interline differences.

TABLE 4.—*Analysis of variance of shank length (restricted population) for each of the different years*

Source of variance	1938		1939		1940		1941		1942	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Between lines			1	4.44	1	3.69	1	34.21	1	45.37
Within lines			322	.188	178	.151	405	.168	340	.177
Total			323	.201	179	.171	406	.251	341	.310
Size line:										
Between sires			1	4.37	2	.370	1	.67	2	1.105
Within sires			82	.144	56	.116	59	.209	83	.222
Between dams			9	.204	6	.033	8	.274	8	.378
Within dams			73	.136	50	.126	51	.199	75	.206
Total			83	.194	58	.124	60	.217	85	.243
Production line:										
Between sires	6	0.145	6	.222	4	.076	9	.186	7	.085
Within sires	361	.190	233	.133	116	.143	336	.113	248	.136
Between dams	35	.289	26	.393	12	.255	37	.340	27	.263
Within dams	326	.180	207	.101	104	.130	299	.085	221	.120
Total	367	.211	239	.185	120	.164	345	.159	255	.155

The average variance within lines remained fairly constant; that within the size line showed a moderate but irregular increase; that

within the production line decreased in every year from the variance of the previous year. It is possible that this reduction in variance is due to the elimination of potential breeders from families with extremely long shanks by incorporating them into the size line, although, as may be seen from table 6, the decrease is not in the interfamily portion of the variance.

TABLE 5.—*Distribution of percentage variance of shank length (restricted population) for the years 1939–42*

Source of variance	1939	1940	1941	1942
	Percent	*Percent*	*Percent*	*Percent*
Between lines	6.5	11.7	33.1	42.9
Within lines:				
Between sires	22.4	10.9	19.0	9.7
Between dams	19.1	3.7	20.3	10.1
Remainder	52.0	73.7	27.6	37.3
Total	100.0	100.0	100.0	100.0

The figures for the more specific sources of variance are best examined when placed on a percentage basis. They appear for the total population in table 5, and for each line separately in table 6. There is a considerable amount of variation from year to year in the portions of variance attributable to different sources. This is undoubtedly due to sampling fluctuations, because the number of parents in each generation is small. Certain general trends may, however, be noted. Thus in the total population, as has already been mentioned, the variance between lines shows a great increase. This increase is only in part at the expense of the residual variance.

TABLE 6.—*Distribution of percentage variance of shank length within lines (restricted population) for each of the different years*

Line	Interfamily genetic variance—	1938	1939	1940	1941	1942
		Percent	*Percent*	*Percent*	*Percent*	*Percent*
Size	Attributable to sire		24.3	0	3.5	8.0
	Attributable to dam		5.6	0	4.8	7.2
	Total		29.9	0*	8.3	15.2
Production	Attributable to sire	9.4	13.7	11.6	21.7	10.8
	Attributable to dam	5.3	31.7	9.1	24.8	11.8
	Total	14.7	45.4	20.7	46.5	22.6

*Mean squares within dams within sires greater than mean error for total. The reduction in mean squares within sires from that of the total is 6.5 percent.

The amount of interfamily genetic variance within the size line has decreased somewhat from the 29.9 percent observed in the first selected generation. There still is, however, at least half of the original variance present. Because of the sampling fluctuations it is impossible to gage accurately what this means in terms of further progress in increasing shank length.

In the production line the amount of interfamily genetic variance fluctuates greatly. It is, however, likely that the genetic variability in this line has not changed significantly from what it was at the beginning of the experiment.

Comparison of the contributions made by the dams with those made by the sire indicates a rather wide range of fluctuation. This may be

due in part to unwitting nonrandom mating within each line, and in part to the small number of parents. On the whole, however, it may be concluded that the contributions of the two parents are nearly equal. This substantiates the finding from reciprocal crosses that neither sex-linkage nor maternal influence are of great importance in the inheritance of size in this material.

It may be recalled that the total genetic variance in the size line as computed by the intrasire daughter-on-dam regression method was 38 percent. The average interfamily heritability in the same population is about 15 percent. The difference between these figures may be considered to represent the average intrafamily genetic variance. Undoubtedly because of the increasing homozygosity of the size line the true value of this constant is higher in the early generations of selection and lower in the later generations. The small numbers preclude the possibility of making such estimates for each year separately. Therefore judgment on the extent of the reduction of the intrafamily part of the variance must be reserved. It is clear, however, that (1) shank length is highly hereditary, and (2) the possibility of further increases in the average shank length by selection in the flock has not been yet exhausted.

SUMMARY

A strain of Leghorns characterized by larger body size, as measured by mature shank length, has been established by selection from a production-bred flock.

The method of selection employed involved progeny and sister testing and led to continued increase in shank length for four generations. The range of individual shank lengths, however, was not extended beyond that of the original flock.

Reciprocal crosses between birds from the line selected for size and birds from the production-bred flock failed to reveal either sex-linked factors or any maternal influence.

Analyses of variance of shank length in the total population for each generation indicated an increase of the portion of variance attributable to the effects of selection, rising to over 40 percent of the total variance in the last generation.

The size line still has a considerable reservoir of interfamily genetic variability, pointing to the possibility of further increases in shank length by family selection.

Estimates of genetic variance of shank length within the Leghorn breed indicate that this character is highly hereditary.

LITERATURE CITED

(1) Burmester, B. R., and Lerner, I. M.
1937. A measuring device for shank length of living birds. Poultry Sci. 16: 211–212, illus.

(2) Bywaters, J. H.
1937. The hereditary and environmental portions of the variance in weaning weights of Poland-China pigs. Genetics 22: 457–468, illus.

(3) Dunn, L. C.
1928. The effect of inbreeding on the bones of the fowl. Conn. (Storrs) Agr. Expt. Sta. Bul. 152: 55–112.

(4) Goldschmidt, R.
1913. Zuchtversuche mit enten. I. Ztschr. Indukt. Abstam. u. Vererbungsl. 9: 161–191.

(5) GOODALE, H. D.
1938. A STUDY OF THE INHERITANCE OF BODY WEIGHT IN THE ALBINO MOUSE BY SELECTION. Jour. Hered. 29: 101–112, illus.
(6) ———
1941. PROGRESS REPORT ON POSSIBILITIES IN PROGENY-TEST BREEDING. Science 94: 442–443, illus.
(7) GOWEN, J. W.
1933. ON THE GENETIC CONSTITUTION OF JERSEY CATTLE AS INFLUENCED BY INHERITANCE AND ENVIRONMENT. Genetics 18: 415–440, illus.
(8) JULL, M. A., and QUINN, J. P.
1931. THE INHERITANCE OF BODY WEIGHT IN THE DOMESTIC FOWL. Jour. Hered. 22: 283–294, illus.
(9) LERNER, I. M.
1937. RELATIVE GROWTH AND HEREDITARY SIZE LIMITATION IN THE DOMESTIC FOWL. Hilgardia 10: 511–560, illus.
(10) ———
1937. SHANK LENGTH AS A CRITERION OF INHERENT SIZE. Poultry Sci. 16: 213–215, illus.
(11) ———
1941. RELATIVE GROWTH IN BANTAMS AND LEGHORNS. Growth 5: 1–10.
(12) ——— and ASMUNDSON, V. S.
1938. GENETIC GROWTH CONSTANTS IN DOMESTIC FOWL. Poultry Sci. 17: 286–294.
(13) LUSH, J. L.
1940. INTRA-SIRE CORRELATIONS OR REGRESSIONS OF OFFSPRING ON DAM AS A METHOD OF ESTIMATING HERITABILITY OF CHARACTERISTICS. Amer. Soc. Anim. Prod. Proc. 1940: 293–301, illus.
(14) ———, HETZER, H. O. and CULBERTSON, G. C.
1934. FACTORS AFFECTING BIRTH WEIGHTS OF SWINE. Genetics 19: 329–343.
(15) MATHER, K.
1941. VARIATION AND SELECTION OF POLYGENIC CHARACTERS. Jour. Genet. 41: 159–193, illus.
(16) MAW, A. J. G.
1935. THE INHERITANCE OF SKELETAL DIMENSIONS IN THE DOMESTIC FOWL. Sci. Agric. 16: 85–112, illus.
(17) PHILLIPS, J. C.
1912. SIZE INHERITANCE IN DUCKS. Jour. Exp. Zool. 12: 369–380.
(18) PUNNETT, R. C., and BAILEY, P. G.
1914. ON INHERITANCE OF WEIGHT IN POULTRY. Jour. Genet. 4: 23–39, illus.
(19) QUISENBERRY, J. H., ROBERTS, E. and CARD, L. E.
1941. GENETIC STUDIES OF SKELETAL DIMENSIONS AND THEIR RELATION TO BODY WEIGHT AND EGG PRODUCTION IN THE DOMESTIC FOWL (GALLUS DOMESTICUS). Poultry Sci. 20: 104–120, illus.
(20) UPP, C. W.
1928. EGG WEIGHT, DAY OLD CHICK WEIGHT AND RATE OF GROWTH IN SINGLE COMB RHODE ISLAND RED CHICKS. Poultry Sci. 7: 151–155.
(21) WALTON, A. and HAMMOND, J.
1938. THE MATERNAL EFFECTS ON GROWTH AND CONFORMATION IN SHIRE HORSE-SHETLAND PONY CROSSES. Roy. Soc. Lond., Proc., Ser. B, 125: 311–335. illus.
(22) WATERS, N. F.
1931. INHERITANCE OF BODY-WEIGHT IN DOMESTIC FOWL. R. I. Agr. Expt. Sta. Bull. 228: 1–105 pp., illus.
(23) WEXELSON, H.
1937. SIZE INHERITANCE IN PIGEONS. Jour. Expt. Zool., 76: 161–186, illus.
(24) WHATLEY, J. A., JR.
1942. INFLUENCE OF HEREDITY AND OTHER FACTORS ON 180-DAY WEIGHT IN POLAND CHINA SWINE. Jour. Agr. Res. 65: 249–264, illus.
(25) WRIGHT, S.
1932. ON THE EVALUATION OF DAIRY SIRES. Amer. Soc. Anim. Prod. Proc. 1931: 71–78.

LENGTH-OF-DAY BEHAVIOR OF NICOTIANA GOSSEI[1]

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INTRODUCTION

On June 21, 1939, seed of a native Australian species of tobacco, *Nicotiana gossei* Domin. (fig. 1), was sent to the writer by Dr. B. T. Dickson, chief of the Division of Plant Industry, Canberra, Australia. The seed was collected by a native connected with the Finke River Mission Station, Hermannsburg, Northern Territory, Australia, about latitude 24° S.

EXPERIMENTAL DATA

To determine the response of *Nicotiana gossei* to length of day plants were first grown in the greenhouse during the winter of 1939, with artificial light to lengthen the short daylight period of winter. In this test, light from one 200-watt, 120-volt, clear Mazda bulb with R L M dome reflector was used from sunset until midnight, affording about 18 hours of unbroken illumination each day. This light suspended about 18 inches above the plants supplied an intensity of 200 to 250 foot-candles.

Seed was sown August 24, 1939. Germination occurred October 5. The tiny plants were pricked off and placed in 4-inch pots, on November 28, then transferred to 6-inch pots January 12, 1940, when the tests began. The results of the tests are shown in table 1.

TABLE 1.—*Responses of Nicotiana gossei to the natural short days of winter in the greenhouse and to supplemental electric light from sunset to extend daily light periods to 18 hours*

[Seed sown August 24, 1939; tests begun Jan. 12, 1940]

Light period and plant No.	Budded	Flowered	Time required to flower	Height
			Days	*Inches*
Natural day (10–11 hours):				
1	February 26	March 7	55	44
2	March 1	March 15	63	34
18 hours:				
1	February 12	February 23	42	42
2	do	do	42	42

Under the conditions of these tests it was evident that with the added light the internodes were shorter and the leaves more numerous and that flowering occurred 2 or 3 weeks earlier than for plants that experienced the natural length of day of wintertime. Since the light conditions of winter in the Washington region are rather unfavorable, tests were continued under the stronger summer sunlight alone.

[1] Received for publication March 3, 1943.

FIGURE 1.—Inflorescence of *Nicotiana gossei*, about one-half natural size. Photographed March 6, 1940.

During the summer of 1940, tests were carried out under natural daylight for all the photoperiods except that of 18 hours. To obtain daylight periods shorter than the full length of day at Washington, D. C., ventilated lightproof dark houses were used. The plants were carried on movable trucks and run into these darkened houses on definite schedules each day to obtain the desired control of daylight exposures.

Since the longest natural day from sunrise to sunset at Washington, D. C., is only 14.9 hours, in order to obtain 18 hours of continuous light each day the plants were exposed, from sunset, to Mazda electric lights from four 200-watt, 220-volt, clear, gas-filled tungsten bulbs with R L M reflectors. These four lights were mounted, one at each corner of a movable square metal frame, so that they were 3 feet apart from center to center of the sides of the frame. This arrangement supplied a light intensity of 300 to 400 foot-candles, as measured by a Weston illumination meter, model 1746, equipped with a Viscor filter to obtain only visible radiation.

TABLE 2.—*Responses of Nicotiana gossei to different daily light periods under natural summer daylight and supplemental electric light*

[Seed sown April 26, 1940; tests begun June 20]

Light period and plant No.	Budded	Flowered	Time required to flower	Height	Leaves to first flower branch
			Days	*Inches*	*Number*
10 hours	July 31	September 20	92	36	32
12 hours	July 12	July 23	33	32	18
13 hours	do	July 29	39	37	26
14 hours	July 1	July 23	33	40	15
18 hours:					
1	July 6	July 17	27	22	12
2	do	do	27	22	14
Full day:					
1	July 17	July 26	40	40	17
2	do	do	40	40	17

Seeds were sown in flats April 26 and germinated May 7. The plants were pricked off into thumb pots June 11 and placed on the tests in 14-quart galvanized buckets June 20, when rosettes were 2 inches high. The results of these tests are shown in table 2.

DISCUSSION AND CONCLUSIONS

From the data of table 1, it will be seen that for the two plants exposed to the short natural days of wintertime an average of 59 days from the beginning of the tests was required for flowering, whereas only 42 days were required for plants receiving 18 hours of light each day.

From table 2 it is seen that the shortened period of 10 hours, where only the summer sunlight was used, delayed flowering until September 20, a period of 92 days from the beginning of the test, June 20. Under the 18-hour photoperiod of this series, flowering took place within 27 days; under the full length of day, flowering took place within 40 days; while the photoperiods of 12, 13, and 14 hours allowed flowering to occur within an average of 35 days (figs. 2 and 3).

The delay in flowering shown by *Nicotiana gossei*, in reality a species of long-day constitution, as shown by the previous tests, would

indicate that the species, although now confined to Australia near latitude 24° S., is not necessarily so confined by the day-length factor. Although the maximum length of day there is not more than 13.6

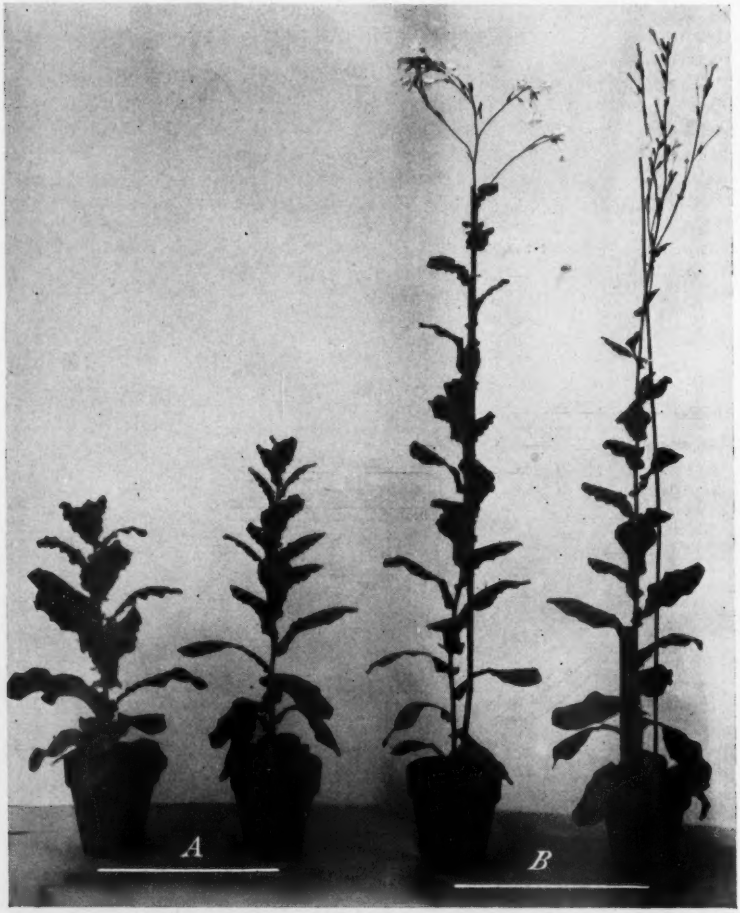

FIGURE 2.—*Nicotiana gossei* grown in the greenhouse at the Arlington Experiment Farm, Arlington, Va., during the winter of 1939–40. Seed was sown August 24, 1939, and tests were begun January 12, 1940. *A*, The two plants that were grown in response to the normal short winter day. These plants flowered March 7 and 15, 55 and 63 days after the tests were begun. *B*, The two plants that were afforded electric light from sunset, giving a constant daily light period of 18 hours. These flowered February 23, 42 days after the tests were begun. Photographed March 6, 1940.

hours, on June 21, this is long enough to induce early flowering, as the tests indicate. The short winter days of its native home, about 10.7 hours on December 21, would be expected to delay flowering.

The tendency of *Nicotiana gossei* to hasten flowering in response to long days and to show retarded flowering in response to short days is strongly in contrast with the short-day behavior of the Maryland Mammoth strain of *Nicotiana tabacum*. The genus *Nicotiana* is mainly a group of day-neutral species that flower readily whether the days are long or short. With the exception of the somewhat anomalous Maryland Mammoth strain and a few others showing similar behavior, the writer has found all the commercial varieties of *Nicotiana tabacum* studied to be day-neutral in their flowering requirements. Likewise the species *Nicotiana rustica* may prove to be a day-neutral assemblage.

It is of interest to note that, were it not for ocean barriers, *Nicotiana gossei*, on the basis of its length-of-day requirements, could well have

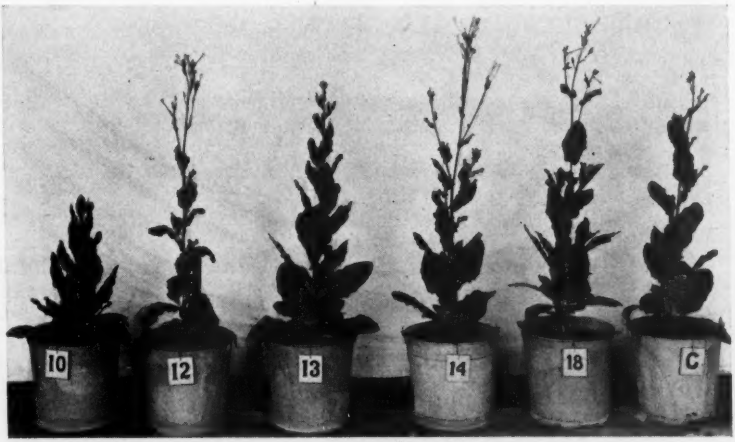

FIGURE 3.—*Nicotiana gossei* grown under different photoperiods during the summer of 1940 at Arlington, Va. Seed was sown April 26, and tests were begun June 20. The plants responded to the various day lengths as follows: 10-hour day, budded July 31, flowered September 20 at 36 inches; 12-hour day, budded July 12, flowered July 23 at 32 inches; 13-hour day, budded July 12, flowered July 29 at 37 inches; 14-hour day, budded July 1, flowered July 23 at 40 inches; 18-hour day, budded July 6, flowered July 17 at 22 inches; full day (*C*), budded July 17, flowered July 26 at 40 inches. Photographed July 25.

become a continental species with a range into much higher latitudes, provided other climatic and habitat conditions were favorable to its survival.

Maryland Mammoth tobacco, on the other hand, represents the opposite tendency, and is better adapted to a distribution into lower latitudes if its natural survival depends upon successful seed production before frost. This inherent requirement of the Maryland Mammoth variety for shortened days has called for special methods of handling and, in order to obtain seed to preserve the strain from extinction, various expedients have been resorted to. The rootstocks in some instances have been carried into the greenhouse, where flowering readily takes place in response to the short winter days, or the plants have been grown in movable containers and given artificially shortened days in darkened rooms or cellars. Lastly, the plants have been grown

in more southern latitudes of the United States, where they can flower naturally out of doors before destructive frosts occur.

It may not be irrelevant to state here that seeds of *Nicotiana gossei* show a very pronounced rest period after harvest, before germination takes place.

Seed harvested and sown thickly on the same day, September 6, showed only a single germinated plant on October 10, whereas seed harvested in the greenhouse March 19 and sown September 6 showed 13 plants germinated and others appearing on October 10. The seed of March 19, although sown at intervals throughout the summer, gave no germination until the sowing of September 6 was made.

SUMMARY

The flowering of *Nicotiana gossei* Domin., seed of which was obtained from Northern Territory, Australia, is favored by long days and delayed by short days.

Artificial light supplementing the naturally short days of the wintertime at the Arlington Experiment Farm, Arlington, Va., greatly hastened flowering.

Reducing the daily light exposures in summertime by means of darkened houses delayed flowering.

The seeds of *Nicotiana gossei* require a long rest period before vigorous germination can occur.

A majority of the varieties of *Nicotiana tabacum* are day-neutral plants, and the Maryland Mammoth variety and a few other Mammoth strains are the only ones in this assemblage at present known definitely to require short days for flowering.

Nicotiana gossei, on the other hand, so far as known at the present time, is the only species in which flowering is very noticeably hastened by long days.

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